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Progress Report ...

Eastern Marketing and Nutrition Research Division

July 1, 1971

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Issued January 1972

PROGRESS REPORT
OF THE
EASTERN MARKETING AND NUTRITION RESEARCH DIVISION
July 1, 1971

INTRODUCTION

The Eastern Marketing and Nutrition Research Division is one of five Divisions of the Agricultural Research Service concerned with developing new and expanded markets for farm commodities. Objectives of the Research program include (1) developing new and improved products and processes to enhance use of the commodities in domestic and foreign markets, (2) improving the safety and nutritive quality of food and feed supplies, and (3) preserving environmental quality by alleviation of soil, water and air pollution.

During the past year this Division conducted research on dairy products, meat, animal fats, hides and leather, Eastern fruits and vegetables including potatoes, tobacco and maple sap and sirup. This report is organized according to these commodities and to areas of research pertinent to each commodity. A list of recent publications and patents is included with each commodity report.

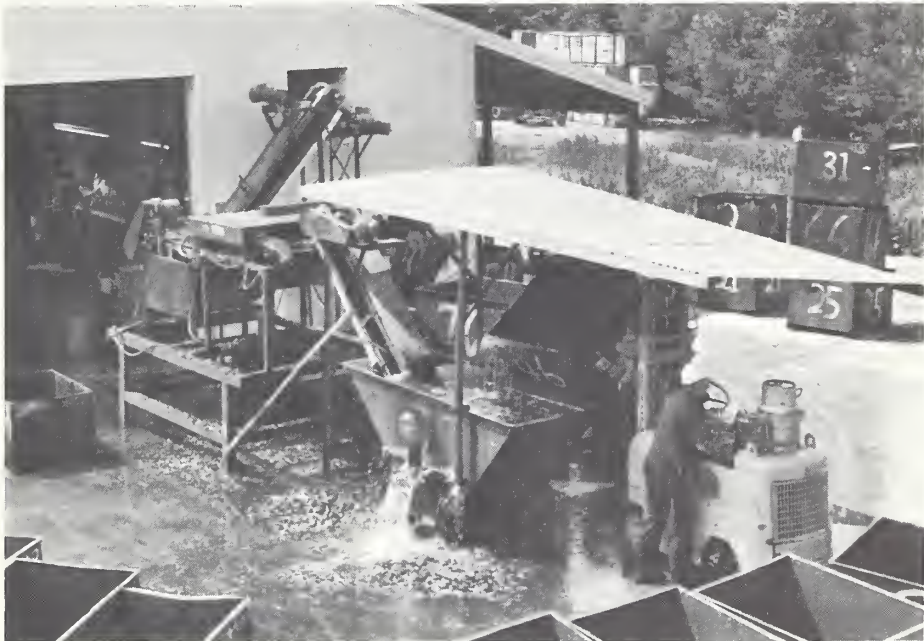
Most of the research is performed in Laboratories located in the headquarters building at Wyndmoor (Philadelphia 19118), Pennsylvania. Exceptions are the Dairy Products Laboratory located in the South Building, U. S. Department of Agriculture, Washington, D.C. and the Agricultural Research Center, Beltsville, Maryland; the Potato Products Investigations in East Grand Forks, Minnesota; and the Pioneering Research Laboratory of Physical Biochemistry at Brandeis University, Waltham, Massachusetts. The other Marketing and Nutrition Divisions are the Northern at Peoria, Illinois, the Southeastern at Athens, Georgia, the Southern at New Orleans, Louisiana, and the Western at Albany (Berkeley 94710), California. In their research, Division scientists cooperate with representatives of colleges and universities, State experiment stations, research institutes and associations, industrial organizations and with other Government agencies. Much of the cooperation is informal, but some work is conducted under conditions described in written cooperative agreements and memorandums of understanding. In addition, the Division supervises domestic research contracts and grants, and the program is supplemented by a variety of research projects in foreign countries under Public Law 480 grants. Division scientists have already achieved much both in terms of discoveries now commercialized and discoveries of a fundamental nature that will be exploited in the future. Following are some examples of recent developments based on research at the Eastern Marketing and Nutrition Research Division that typify such achievements.

SELECTED EXAMPLES

ON-THE-FARM CHERRY PROCESSING

In the past, cherries have been collected at the orchard and shipped to central packing plants for processing. This practice can result in losses in product quality due to hauling, rehandling and delays arising from production scheduling at the plant. A team of Department scientists of different disciplines studied this problem and demonstrated that processing at the orchard site is practical and that the problems associated with such a practice could be overcome. The team prepared specific recommendations for industry on how this could be accomplished. Last season, a number of tart cherry producers in the Michigan cherry producing region adopted the recommendations, and reported that the new procedure is very successful. Using rented equipment, eight orchard-side plants handled 15% of the national crop and achieved a level of product quality not previously attained. As a result, additional gross income of about \$450,000 was realized by these growers. The increase in yield of processed fruit added \$270,000 and the increase in fruit quality contributed \$180,000 to this additional income. The success of this development is now firmly established and is serving as the initial step in reversing the recent trend toward large, centralized cherry processing operations, with resulting benefit to rural communities.

ON - THE - FARM CHERRY PROCESSING



- ☆ ***CENTRALIZED PROCESSING GAVE EXCESSIVE LOSSES AND LOW QUALITY DUE TO SHIPPING AND HANDLING.***
- ☆ ***RESEARCH SHOWED ON-THE-FARM PROCESSING TO BE PRACTICAL.***
- ☆ ***GROWERS ADOPTED NEW PROCEDURE WITH: REDUCED LOSSES, IMPROVED QUALITY, LARGER INCOME.***

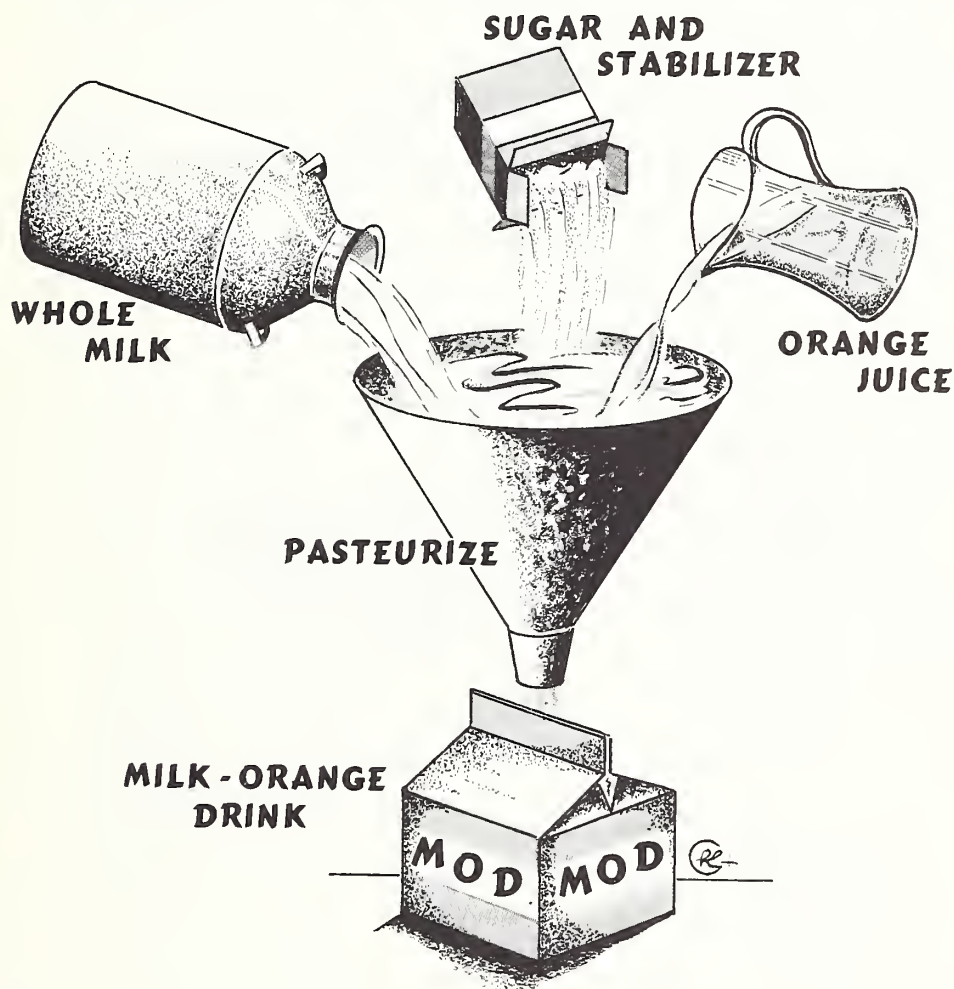
IMPORTANT TO RURAL COMMUNITIES

MILK-ORANGE JUICE BEVERAGE DEVELOPED

The continuing decline in per capita consumption of whole milk is of considerable concern in relation to the nutritional well-being of the U. S. population, and to the economy of the dairy industry. The development of new milk or milk-containing products with appeal to consumers, including non-milk drinkers, would help halt or reverse the decline. Such a product containing milk and orange juice as the principal ingredients has been developed cooperatively by Department scientists and Dairy Development, Inc., a nonprofit corporation owned by dairy cooperatives in the Northeast. The preferred formulation of the product, called "MOD", (from Milk Orange juice Drink), contains about 3 parts of whole milk and 2 parts of orange juice plus sufficient sugar for sweetness and a small amount of stabilizer. This formulation gives a stable, flavorful beverage which cannot be obtained by simple admixture of orange juice and milk. The refreshing drink combines the highly desirable protein and mineral constituents of milk with the vitamins and other nutrients of orange juice. The amount of vitamin C in one 8 oz. glass of the beverage supplies 100% of the recommended daily requirement of that vitamin for children 10 years of age. Tests indicate no significant loss of vitamin C as a result of pasteurization.

The product has been received favorably in consumer tests involving more than 15,000 samples. A market test in one cafeteria showed that 50% of the purchasers bought the beverage a second time. The purchases were made at the expense of other non-carbonated beverages rather than milk. The product has been produced satisfactorily on a commercial scale and is now available for marketing as a new dairy product. Successful marketing of the beverage may stimulate interest in related nutritious products containing mixtures of milk and other fruit juices that could be developed.

MILK - ORANGE JUICE BEVERAGE



- ☆ **NUTRITIOUS PRODUCT—BLENDS PROTEIN AND MINERALS OF MILK WITH VITAMINS AND NUTRIENTS OF ORANGE JUICE.**
- ☆ **EASY PROCESSING—CAN BE MADE IN EXISTING EQUIPMENT IN DAIRIES.**
- ☆ **CONSUMER TESTED—FAVORABLY RECEIVED WITH GOOD REPEAT SALES.**

NEW VEGETABLE AND FRUIT SNACKS

Several new deep-fat fried vegetable and fruit chip products developed by Department scientists have now achieved nationwide marketing, and inquiries from Canada and other countries indicate that international distribution may be imminent. The new snack items combine the natural taste of the vegetables with unusual flavor overtones that form during the processing. The chips have excellent keeping quality. In developing these new products, a study by Department workers indicated that five vegetables could be processed into chip products that had potential for consumer use. Variety and maturity studies were then made for each of these vegetables in order to select the best raw material for processing. Samples of chips were produced under various processing conditions and were evaluated for consumer acceptance, with emphasis on flavor and texture. Optimum conditions were then chosen for the manufacturing of the chips. Later, the work was extended to the processing of apples into chips and again an acceptable product was developed. At present, chips are being produced commercially from apples, sweet potatoes, beets, mushrooms and parsnips. Since only the highest quality raw material is used to produce an acceptable snack food, both the grower and the consumer benefit by this development. An estimate of the retail value of current production is over \$400,000.

DELICIOUS....

***CRISPY VEGETABLE AND FRUIT
SNACKS PROVIDE TASTE TREAT.***



☆ ***NATURAL FLAVOR ENHANCED BY FRENCH FRYING.***

☆ ***APPLE, SWEET POTATO, BEET, MUSHROOM AND
PARSNIP CHIPS NOW AVAILABLE COMMERCIALY.***

PHOSPHATE-FREE DETERGENT FORMULATIONS

Household and industrial detergents must be formulated so that their subsequent disposal into water systems will not have an adverse effect on the native plant and animal life in these waters. One way that detergents may produce an adverse effect is by stimulating the growth of certain plants, which eventually results in a disturbance of the ecological balance of aquatic life. It is believed by many that the phosphates in detergents act in this way by stimulating the growth of algae; eventually, the excessive algal growth decomposes and, in the process, depletes the oxygen in the water, a process called "eutrophication". Many aquatic plants and animals, including fish, cannot survive in such oxygen-depleted water.

Although detergents without phosphates are currently on the market, many possess serious shortcomings and are not universally acceptable. Department scientists are now developing phosphate-less detergents based on soap, which do not have these serious shortcomings. Soap is an excellent detergent but has the disadvantage of producing a curd ("bath-tub ring") with water of even moderate hardness. This curd is esthetically undesirable and, during washing, is deposited on clothes, giving them a grayish cast. Department workers have found that, by adding certain chemicals derived from animal fats, soap can exert its excellent cleaning power without curd formation. These chemicals are known as "lime soap dispersing agents". In laboratory tests, combinations of soap and lime soap dispersing agents without phosphates can be formulated that give cleaning power excelling the common, highly effective phosphate-containing household detergents even in hard water. These results show the feasibility of developing effective household cleaners that do not contribute to water pollution.

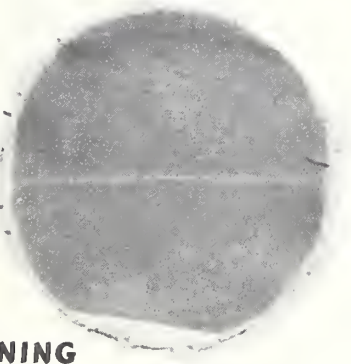
NEW PHOSPHATE - LESS DETERGENTS

SOAP PLUS CURD DISPERSANT

NO DISPERSANT



**HEAVY
CURD**



**LESS
CLEANING**

WITH DISPERSANT



**CURD
DISPERSED**



**BETTER
CLEANING**

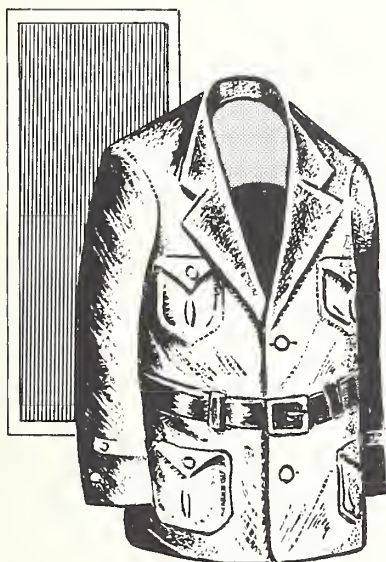
- ☆ SOAP PLUS DISPERSANT EFFECTIVE IN HARD WATER.
- ☆ EXCELS PHOSPHATE DETERGENT IN CLEANING.
- ☆ HELPS ELIMINATE WATER POLLUTION.

NEW PROCESS FOR DRY CLEANABLE LEATHER

Garments made from leather are becoming increasingly popular. A major shortcoming of such garments is the lack of an inexpensive method for cleaning. Ideally, such garments should be capable of being cleaned by common dry cleaning methods without special handling; however, until recently, these cleaning methods altered the properties of the leather since the solvent leached the fat liquor from the leather and the garments lost their original flexibility and suppleness. To restore these properties it was necessary to reprocess the garments after dry cleaning, which greatly increased the cleaning cost.

Department scientists have now developed a way to treat leather so that it can be dry cleaned. During processing of the leather and prior to manufacturing into garments, the leather is treated with a special chemical substance, a long chain amino acid derivative made from coconut oil. This agent acts as a fat liquoring agent which is resistant to dry cleaning solvents. No re-oiling or other processing is needed after the dry cleaning. Samples of suede leather have been produced in a commercial tannery using this process and the samples possess a high degree of dry cleanability.

DRY CLEANABLE LEATHER



☆ OLD METHOD—
REQUIRED REPROCESSING
OF LEATHER AFTER
CLEANING.

☆ NEW METHOD—
NO REPROCESSING
NEEDED.

☆ CUTS COST OF DRY CLEAN-
ING LEATHER.

**SOLVES AN IMPORTANT PROBLEM IN EXPANDING
MARKET FOR LEATHER GARMENTS.**

DAIRY PRODUCTS UTILIZATION

Problems and Objectives

Although consumption of low fat milk has increased, the per capita milk equivalent of all fluid items has shown a downward trend for more than 10 years. These decreases have adverse implications to nutrition of the general population and to economic stability of the dairy industry. Butter remains in excess and cheese whey is a serious stream pollution problem. Research on utilization of milk emphasizes the development of better processing procedures; improved dairy products, including more stable and easily transported products; new and increased food uses, especially for those milk components in excess supply; and economical disposal of dairy wastes.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Develop milk products of enhanced nutritional properties.
2. Develop dry whole and concentrated milks of beverage quality.
3. Prevent the formation of or eliminate objectionable flavors in dairy products, and stabilize and intensify desirable flavors.
4. Prevent or eliminate contamination of dairy products with disease-producing microorganisms.
5. Develop food uses and more economical disposal processes for cheese whey.
6. Develop new cheese and milk fat products for food use.
7. Determine the effects of processing and storage on the nutrient composition of natural and enriched dairy products.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED MILK PRODUCTS

Fluid Milk Products. Sterilized whipping creams of various compositions were made to contain stabilizers and emulsifiers to control heat and storage stability and whipping properties. These creams remained whippable with little if any fat separation for more than 6 months at 5°C, while creams without additives formed solid cream layers. The

whippability of the creams (whipping time, drainage, overrun and stiffness) was affected by the percentage of fat, type and concentration of stabilizer and by homogenizing pressure and sequence. Drainage was eliminated by the use of 0.015% carrageenan in the 32% fat and 0.025% in the 26% fat containing creams. Whipping times of creams containing stabilizers were approximately 100 sec. for the 32% fat and up to 240 sec. for the 26% fat product. The best body was obtained in a product containing 32% fat, 0.1% sodium alginate and 0.015% carrageenan. This product whipped to 155% overrun in 120 sec. with no significant drainage. Similar results were obtained using only xanthan gum except that the whip was softer.

The occurrence of several aromatic acids as normal constituents of raw milk has been established. The role of benzoic, phenylacetic and cinnamic acids as precursors to several highly flavorful aromatic aldehydes and esters, reported to arise during the flavor deterioration of sterile milks, is suggested.

In other flavor research, further insight into the possible involvement of Maillard browning in staling of dairy products has been gained by the study of whey. Gas chromatographic and mass spectral analysis of a steam volatile oil obtained from commercial spray-dried whey resulted in the identification of 24 compounds, including 7 alkylpyrazines. The nature of some of the identified products indicates that the browning reaction proceeded to a significant extent during manufacture and storage, and suggests that the processing conditions favor the Strecker degradation. Other results indicate that lactose could be one of the precursors of the oxidized flavor. The latter finding, if confirmed, will affect the formulation of recommendations to control the development of the oxidized flavor in dairy products.

Biochemical studies of sporulation of Bacillus species are in progress at U. P. Agricultural University, Pantnagar, India, under a PL-480 grant. The production of heat sensitive spores with significant content of dipicolinic acid by growth and sporulation in synthetic media containing nicotinamide, picolinamide and ethyl oxamate may prove useful in unravelling the mechanism of heat resistance. In research at EMN a disulfide reductase from Bacillus cereus T spores was purified 200-fold, and was shown to possess thioredoxin reductase activity. Further, spore extracts were found to contain thioredoxin. Thus, it was demonstrated that dormant spores of Bacillus cereus T possess the reducing capacity to support biosynthesis of deoxyribonucleotides which are vital to DNA synthesis during germination.

A heat-labile nondialyzable factor, discovered in Bacillus cereus T spore extracts, stimulated the activity of sporolytic enzymes. The new factor possesses no glycosidase activity.

Small angle X-ray scattering has shown that the α -lactalbumin of milk is indistinguishable from hen's egg white lysozyme in its hydrodynamic parameters. Such similarities have been intimated from extensive homologies in primary structure.

β -Lactoglobulin contains about 0.1% of lipid which cannot be removed by repeated crystallization. Removal of the lipid by charcoal extraction renders the protein insoluble and subject to denaturation. Recrystallization of β -lactoglobulin with added butterfat gives a protein which is significantly more associable at low temperature than conventionally prepared globulin.

Molecular weight studies on the minor proteins (γ -, TS-, R-, S-) add credence to the concept that these proteins are degradation products of β -casein.

The gelation process in concentrated skim milk was shown to begin by lateral aggregation and "bridging" of individual micelles after 9 weeks' storage with essentially complete gelation after 17 weeks.

Differences in amino acid sequences responsible for property differences among the various α_{s1} -casein polymorphs were determined and found to result in changes in the relative hydrophobicity of the molecules.

Lactate dehydrogenase, prepared from beef heart and dogfish, consists of four subunits; literature reports indicating eight subunits for the molecule are in error. The molecular weight of calf brain microtubule is 55,000; the stable unit is a dimer.

Ribonuclease excludes 2-methyl-2,4-pentanediol from its environment and is preferentially hydrated in water-organic solvent mixtures. The absolute binding of organic solvent in mixtures of 2-chloroethanol with water is proportional to the hydrophobicity of the protein.

Under a grant at Oklahoma State University, Stillwater, the A protein of lactose synthetase has been fractionated into three enzymatically active components. It contains two carbohydrate chains containing sialic acid, mannose, galactose, fucose, N-acetyl-glucosamine and N-acetylgalactosamine. The kinetics of the lactose synthetase reaction indicates that reagents add sequentially in the order UDP-galactose, glucose and α -lactalbumin.

In research under a PL-480 grant at Israel Institute of Technology, Haifa, 5-fluorouracil was shown to selectively inhibit messenger RNA synthesis (and consequently protein synthesis) in virus-infected bacteria; however, the pyrimidine analogue proved to be a general ribosomal protein inhibitor

in bacteria which had not been infected with virus. Studies on the mechanism of 5-fluorouracil-induced resistance to ultraviolet light in bacteria showed the compound as having an indirect effect. Effect of ultraviolet irradiation of DNA in the presence and absence of photosensitizers was studied, and selectivity in terms of alteration of purine and pyrimidine bases was achieved by modifying the conditions of irradiation.

Dry Milk Products. A storage stable powder that easily reconstitutes to a beverage almost indistinguishable from fresh, pasteurized whole milk has been made by mixing steam deodorized butteroil with skim milk, homogenizing the mixture and then submitting it to conventional pasteurization, condensing and foam spray drying. The deodorizing step must be controlled to remove off-flavor precursors without loss of milk flavor. The powder, when packed with an oxygen scavenger, will keep at 80°F for at least 6 months without significant flavor change. The modifications in conventional spray dried whole milk manufacture necessary to achieve this product stability are relatively inexpensive and should require an "add on" cost of approximately 1/2 cent per quart equivalent.

In the vacuum drying process the feed nozzle for applying the concentrated whole milk was observed to operate satisfactorily at simulated five times the pilot plant feed rate without requiring significant design modification. However, it was demonstrated that a holding section for the gassed concentrate, not previously shown in published information, must be provided in order to establish a satisfactory foam blanket for drying. This hold section should be inserted immediately prior to introducing the feed-to-the-dehydrator into the final temperature adjustment heat exchanger. The scale-up ratio used to estimate commercial costs from pilot plant data was confirmed.

Data pertaining to the organoleptic properties of fresh and stored whole milk powders and butteroils have been correlated with the GLC patterns obtained by analysis of the steam distillates from the fat in those products. Quality control now becomes possible by using computer techniques to locate those peaks in the complex patterns whose variance has been found to be related to product flavor change in a significant fashion.

In a part of the continuing investigation of components of dairy products related to flavor, a quantitative method for estimating dihydric alcohols as bis-esters of pyruvic acid chloride 2,6-dinitrophenylhydrazones and their isolation from monohydric alcohols which are simultaneously quantitated was developed. Other methods developed include: regeneration of 2,4-dinitrophenylhydrazones on a periodic acid column; detection of unsaturation in submicrogram amounts of colored derivatives of alcohols, amines, carbonyls and fatty acids; location of double bond position in

monoolefins; fractionation of fatty acids on buffered celite columns; fractionation of amines on buffered celite columns; and determination of hydroxy fatty acid content of lipids.

The gelation of milk, with subsequent loss in protein solubility, as observed in concentrates having total solids higher than 50% and in conventionally spray dried milk powder during reconstitution, results from the elimination of κ -casein from the milk micelle by the action of the naturally occurring milk phosphates.

The heat applied to milk and whey during spray drying does not significantly change the amount of total lysine in the products or its biological availability as measured by chemical methods. Roller drying was found to significantly reduce and render unavailable the lysine found in the proteins of cheese wheys.

The lactose in dried whey products was found to suppress bread loaf volume, primarily by reducing yeast activity. This effect can be circumvented by using dry yeast and proofing to height instead of to time.

Standard antioxidants sometimes considered for use in dehydrated products were found to inhibit the physiological action of bradykinin and a similar acting, but as yet uncharacterized, kinin present in milk and some dairy products.

The minor caseins termed γ -, TS-, R- and S- have been shown to be fragments of β -casein. They differ from β -casein in number of amino acids and N-terminal amino acids. They presumably arise from proteolytic degradation of the parent molecule, β -casein.

In PL-480 sponsored research on phosphopeptides of casein at Indian Institute of Science, Bangalore, India, studies on the chymotryptic peptides indicate that the α_{s1} -casein isolated from the pure Jersey breed cow is the genetic variant B. The tryptic digest of α_{s1} -casein has been resolved into 23 peptides and the amino acid composition of 6 tryptic peptides has been determined.

Milk Fat Products. In research at EMN on a continuous process for making ghee, the equipment has been developed to include a holding tube downstream from the scraped surface heater. This addition will make it possible to study the effect of the "time-temperature" relationship upon the product. One exploratory run has been made with pre-stratified butter to test this added feature. A tubular-type cooler has been incorporated downstream from the holding tube and is ready to be tested. Clarification, the final step, will be added downstream from the cooler. Clarification is needed to remove caramelized non-fat solids from the product prior to packaging.

Studies at the National Dairy Research Institute, Karnal, Punjab, India, under a PL-480 grant indicate that ghee clarified at 120°C is scored higher by their flavor test panel than ghee clarified at lower temperatures. Comparison of the total volatiles from ghee with volatiles from isolated neutral, acidic and lactonic fractions suggests that the neutral volatile fraction is the major contributor to the flavor of ghee. Resolution of the neutral fraction indicates that carbonyl compounds are dominant in imparting desirable flavor. Kind and amount of carbonyl compounds in the ghee appear to vary with method of manufacture.

In research at EMN to improve the stability of butterfat, selective hydrogenation of milk fat, resulting in an almost total elimination of C₁₈₌₃ fatty acid, was achieved with commercially available copper catalysts. Taste panel evaluation of the hydrogenated fat indicated a strong objectionable foreign flavor. Milk fat fractionation by zone refining was effected only in the higher melting triglyceride range because of limitations in the achievable freezing temperatures.

Cheese and Cultured Milk Products. Supplemental starter microorganisms and their crude enzyme preparations have been used in the Cheddar cheese process with the objectives of accelerating flavor development and increasing rate of ripening. Assays of the stored cheese will be made at monthly intervals.

Under a PL-480 grant at University of Belgrade, Zemun, Yugoslavia, a pure culture collection was established of lactic acid bacteria for use as starter cultures in milk processing. This collection includes 16 Streptococcus species, 24 strains of Lactobacterium and 80 strains of Micrococcus species. While the majority were isolated from cheeses, a few were obtained from sausages, air and other media. Preliminary investigations have demonstrated the advantages of the use of mixed cultures. For example, a micrococci which did not form diacetyl as a pure culture in milk stimulated an abundant formation of diacetyl in mixed populations with Str. thermophilus. Also, a Str. thermophilus strain required 3 hours at 40°C to curdle milk, but a mixed culture with a Micrococcus strain achieved a similar result in 2.5 hours.

FOREIGN MARKET DEVELOPMENT

Cheese Whey Blends. Additional plant protein concentrates obtained from laboratory and commercial sources were screened for the bland organoleptic properties which would make their inclusion in whey based beverages feasible. The results were disappointing. No product markedly superior to soy-flour was found. Obviously, more advances must be made before cottonseeds, peanuts, sesame seeds, wheat and soybeans can provide inexpensive protein concentrates that are bland enough to compete with milk

proteins in the beverage field. However, whey-soy blends are already bland enough for inclusion in breads, frozen desserts and citrus flavored beverages.

In supporting research, complete isotherms describing the absorption of water by orange solids, whey solids, soy flour and mixtures of citrus flavored whey and soy proteins were obtained in an attempt to resolve the difficulties encountered in drying acid whey-soy flour mixtures stabilized by gums.

Differential scanning calorimetry was used to determine the amount of bound water in concentrates of whey and soy protein, and the specific heats of purified whey proteins, whey protein mixtures and whey concentrates.

Methods for producing a whey-milk fat spread type product on pilot plant scale were improved to the point that relatively large quantities of high quality material could be consistently made for consumer test purposes. A paper describing the product and detailed methods for its production has been presented publicly and submitted for publication.

Foam spray drying has been routinely used to produce non-hygroscopic acid whey powders from concentrates. Both powders and concentrates contain 70% of the lactose in the crystalline α -form.

PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND NATURALLY OCCURRING TOXINS

Salmonella in Milk Products. A modified detection procedure has been devised incorporating a previously developed lysine-iron medium with fluorescent antibody microscopy to give a one-step selective and enrichment medium which would detect one Salmonella bacterium in 100 g of non-fat dry milk within 24 hrs.

Preliminary results indicate that the extraction procedure for salmonellae lipopolysaccharide yields a biologically potent endotoxin as determined by mouse lethality tests.

Milk Allergens. A total of 12 new antigens have been generated by brief pepsin hydrolysis of milk proteins. One new antigen was demonstrated from each of α -lactalbumin and casein, two from bovine serum albumin and 8 from β -lactoglobulin. These results provide an explanation as to why milk, upon ingestion, produces an immediate-type allergic response in individuals on which a skin test with unhydrolyzed milk protein is negative. Such persons may be sensitive to the new antigens formed in the first stages of digestion. Four new antigens have been demonstrated in lactose

which are distinct from the known milk proteins. These new antigens may account for the reported 100-fold increase in allergenicity of β -lactoglobulin on heating with lactose.

Studies on the identification, classification and function of the bovine immunoglobulin system have been continued with following results: (1) secretory IgA (SIgA) is the principal immunoglobulin synthesized by the secretory tissues of the cow except the mammary gland in which, however, the secretory component (glycoprotein-a) is abundant; (2) an antiserum has been obtained which detects antigenic differences between lacteal SIgA and lacrimal SIgA. This may be the first evidence for IgA subclasses in cattle; (3) there is no correlation between the amount of IgG1 in milk and colostrum with that in the serum of the same cow; (4) the level of IgA in bovine serum varies among individual cows but is positively correlated with the amount of IgA present in the secretions of these animals; (5) SIgA occurs in bovine serum and is at a higher level immediately before calving.

"Purified" milk proteins that have been used for passive transfer studies were examined for purity by means of immunodiffusion (ID), immuno- and disc-electrophoresis (DE). Some contaminants were identified; two were quantitated. β -Lactoglobulin (BLG) was free of contaminants as determined by immunodiffusion, but on DE a faint band appeared at the α -lactalbumin region. α -Lactalbumin and bovine gamma globulin contained 2.3% and 0.4%, respectively, of bovine serum albumin. Ribonuclease A contained a trace of RNase B, but was free of other milk proteins. The mucoprotein showed a faint band on DE in the BLG area, and it reacted with α -casein antiserum - partly due to contamination with BLG and a second unidentified component. The absorbed mucoprotein antiserum was specific. Both α -casein and β -casein showed 5 bands on DE and both reacted with α_{s1} -casein, β -casein CC and γ -casein and their homologous antisera. Thus, either the caseins contain a common, unidentified contaminant, or share antigenic determinants that obstruct individual immunologic recognition. κ -Casein is immunologically distinct from other caseins.

Research on development of specific sensitivity in experimental animals corresponding to allergy in humans was successful in the rat. The antibody responsible for transferring anaphylactic sensitivity to normal rats has been tentatively identified of the IgG_a class of immunoglobulin. The rabbit homocytotropic antibody was not identified. Both the rat and the rabbit are capable of producing more than one homocytotropic antibody.

HUMAN NUTRITION

Nutritional Enrichment of Dairy Products. Research was continued on the enrichment of milk and milk products with iron. A commercially feasible

method for fortifying whole milk involves addition of ferric ammonium citrate followed by pasteurization at 81°C. A temperature level sufficient to inactivate milk lipase is critical to quality. In the presence of ferric compounds, milk lipase increased in heat resistance producing a "rancid" flavor subsequent to pasteurization below 79°C.

Iron polyphosphate (FIP) and a whey protein-iron polyphosphate (FIPP) have been prepared. When FIPP was added to whole milk, the product was equal in flavor score to controls. The protein complex has about the same efficiency as FeSO_4 in restoring hemoglobin levels in rats, and about the same efficiency when added to milk and concentrated milk products. The iron polyphosphates have been used to fortify bread doughs with iron without deleterious effects on flavor, color or loaf volume, and show promise as excellent additives to cereals and baked products.

Ferrous compounds did not cause "rancidity", but induced an "oxidized" flavor when added before pasteurization.

Commercially feasible iron fortified nonfat dried milks were made by adding ferric compounds to skim milk prior to pasteurizing, condensing and spray drying. After reconstitution, the beverage milk contained 22 mg/qt iron. The solubility, initial flavor and storage stability of nonfat dried milk were not impaired by this addition of iron. Addition of ferrous iron to skim milk, at this level, produced undesirable off-flavors. No relationship was found between the storage stability of fluid skim milks containing ferric iron and that of the powders made therefrom. Iron fortified nonfat dried milk was found to be satisfactory for most household cooking uses except for the making of cocoa. Here a marked color shift in the beverage was noted when the iron fortified skim milk was used.

Centrifugal fractionation of iron fortified milk showed that about 80% of the added iron complexes with the caseinate sediment. From 98 to 100% of the added iron is non-dialyzable and assumed to be complexed with proteins.

Changes in the fluorescent spectra of whey proteins on addition of various ferrous and ferric salts were studied to obtain information related to the binding energies involved in the formation of the iron-protein complexes formed in milk. Changes in those spectra reflect changes in the anion of the iron salts and may correlate with either the solubility or assimilability of the iron-protein complexes.

Taste tests were made on whole milks prepared by adding vegetable oils before pasteurization in quantities equivalent to a linoleic acid content

of 5% of the total fat. Corn oil and cottonseed oil gave flavor scores comparable to controls, but soybean and safflower oil were unacceptable.

By feeding cows a ration containing a formaldehyde-treated safflower-casein preparation, the linoleic acid content of milk increased from 3% to 35% of the total fatty acids. Taste tests of pasteurized samples of high linoleic acid milks revealed definite to strong oxidized flavors.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

Whey Disposal by Utilization. Research at EMN on the concentration of whey by reverse osmosis (RO) and fractionation by ultrafiltration (UF) is being adapted to commercial scale processing under a contract with H. P. Hood and Co., Boston, Massachusetts. Preliminary trials have indicated that the RO system was functioning properly but the UF system was not. Critical tests have now located the problem and it is being resolved by replacement of the UF membranes. Accumulation of data to determine commercial feasibility has now begun.

In continuing research at EMN, cottage cheese whey is being processed on a regular basis to give a protein fraction containing about 20% protein. Further purification through Sephadex columns has been demonstrated feasible. Small amounts of protein so isolated and subjected to intensive clarification have been spray dried and lyophilized to produce powders that have excellent solubility at the low pHs. Studies of the rate of whey protein denaturation across a pH range extending from 2.0-4.5 have been made. The maximum stability observed at pH 3.4 makes these proteins excellent prospective agents for the fortification of carbonated beverages and citrus fruit juices.

High protein concentrates containing 35% protein can be diluted and subjected to a second fractionation to give a 65 to 70% protein concentrate having unique functional characteristics. The protein is soluble, whippable and has excellent water binding properties.

A study of the farinograph and baking characteristics of doughs containing whey protein concentrates showed that improved water absorption and dough handling properties were observed when the level of denatured whey protein in the product was high.

The preparation of whey products in which the lactose has been hydrolyzed to its simple sugars may lead to increased utilization. Commercial lactase preparations were evaluated and the β -D-galactosidase of A. niger was purified by affinity chromatography using the lactase inhibitor, p-aminophenyl- β -D-thiogalactopyranoside. The isolated enzyme was covalently coupled by diazo-linkage to porous glass beads and the kinetics of the free and bound enzymes compared. The bound enzyme was adaptable to

continuous column operation, but due to product inhibition, activity levels per gram were inversely related to the volume of the bound enzyme. A column containing 10 cc of the bound enzyme hydrolyzed 33% of the lactose in acid whey at a continuous flow rate of 100 ml/hr. The enzyme has a pH optimum of 4.5 and its use is restricted to products in this pH range. This work is being extended to other lactases having pH optima in the pH range of 6.0 to 7.5.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED MILK PRODUCTS

- Aune, K. C., Goldsmith, L. C. and Timasheff, S. N. The dimerization of α -chymotrypsin. II. Ionic strength and temperature dependence. *Biochemistry* 10 (1971) 1617-1622.
- Aune, K. C. and Timasheff, S. N. The dimerization of α -chymotrypsin. I. pH Dependence in the acid region. *Biochemistry* 10 (1971) 1609-1617.
- Berlin, E., Kliman, P. G. and Pallansch, M. J. Changes in state of water in proteinaceous systems. *J. Colloid & Interface Sci.* 34 (1970) 488-494.
- Berlin, E., Kliman, P. G. and Pallansch, M. J. Calorimetry and thermogravimetry of bound water in dried milk and whey powders. *J. Dairy Sci.* 54 (1971) 300-305.
- Brewington, C. R., Caress, E. A. and Schwartz, D. P. Isolation and identification of new constituents in milk fat. *J. Lipid Research* 11 (1970) 355-361.
- Cerbulis, J. Selective separation of some proteins from milk with fat solvents. *Milchwissenschaft* 25 (1970) 76-78.
- Cornell, D. G., De Vilbiss, E. D. and Pallansch, M. J. Binding of anti-oxidants by milk proteins. *J. Dairy Sci.* 54 (1971) 634-637.
- Ettinger, M. J. and Timasheff, S. N. Optical activity of insulin. I. On the nature of the circular dichroism bands. *Biochemistry* 10 (1971) 824-831.
- Ettinger, M. J. and Timasheff, S. N. Optical activity of insulin. II. Effect of nonaqueous solvents. *Biochemistry* 10 (1971) 831-840.
- Farrell, H. M., Jr., Thompson, M. P. and Larsen, B. Verification of the occurrence of the α_{s1} -casein A allele in red Danish cattle. *J. Dairy Sci.* 54 (1971) 423-425.
- Fasman, G. D., Hoving, H. and Timasheff, S. N. Circular dichroism of polypeptide and protein conformations. Film studies. *Biochemistry* 9 (1970) 3316-3324.
- Ferretti, A. and Flanagan, V. P. The lactose-casein (Maillard) browning system: volatile components. *J. Agr. Food Chem.* 19 (1971) 245-249.

- Fitzgerald, D. K., Colvin, B., Mawal, R. and Ebner, K. E. Enzymic assay for galactosyl transferase activity of lactose synthetase and α -lactalbumin in purified and crude systems. *Anal. Biochem.* 36 (1970) 43-61.
- Fox, K. K. Casein and whey protein in Byproducts from Milk. B. H. Webb and E. O. Whittier (Editors) 2nd ed. pp 331-355. Westport, Conn., Avi Pub. Co. (1970).
- Groves, M. L. and Gordon, W. G. The major component of human casein: A protein phosphorylated at different levels. *Arch. Biochem. Biophys.* 140 (1970) 47-51.
- Groves, M. L. and Kiddy, C. A. γ -Caseins isolated from milk samples typed β -casein A¹ and A³. *J. Dairy Sci.* 53 (1970) 931-933.
- Groves, M. L. and Townend, R. E. Molecular weight of some human and cow caseins. *Arch. Biochem. Biophys.* 139 (1970) 406-409.
- Guy, E. J. Lactose: Review of its properties and uses in bakery products. *Bakers Digest* 45 (2) (1971) 34-36, 38, 74.
- Guy, E. J., Vettel, H. E. and Pallansch, M. J. Effect of lactose in non-fat dry milk and cheese whey solids on sponge bread volume and yeast activity. *Bakers Digest* 45 (3) (1971) 43-44, 46-47, 50-51.
- Holsinger, V. H., Posati, L. P., De Vilbiss, E. D. and Pallansch, M. J. Effect of processing on available lysine content of whey powders. Abstract M83 *J. Dairy Sci.* 54 (1971) 765.
- Holsinger, V. H., Posati, L. P. and Pallansch, M. J. Anomalous results obtained in the determination of available lysine in casein using 2,4,6-trinitrobenzenesulfonic acid. *J. Dairy Sci.* 53 (1970) 1638-1639.
- Huhtanen, C. N., Brazis, A. R., Arledge, W. L., Cook, E. W., Donnelly, C. B., Ginn, R. E., Murphy, J. N., Randolph, H. E., Sing, E. L. and Thompson, D. I. Effect of dilution bottle mixing methods on plate counts of raw-milk bacteria. *J. Milk Food Tech.* 33 (1970) 269-273.
- Jones, S. B. and Kalan, E. B. Modified procedure for isolation of a major swine whey protein. *J. Dairy Sci.* 54 (1971) 288-291.
- Kurtz, F. E., Tamsma, A. and Pallansch, M. J. Organoleptic properties of foam spray-dried products made from deodorized milk fat and skimmilk. *J. Dairy Sci.* 54 (1971) 173-177.

- Posati, L. P., Fox, K. K. and Pallansch, M. J. Inhibition of bradykinin by gallates. J. Agr. Food Chem. 18 (1970) 632-635.
- Sadler, A. M. and Wong, N. P. Milkfat utilization in foods. In Byproducts from Milk. B. H. Webb and E. O. Whittier, Editors, 2nd Ed., pp 274-294. Westport, Conn., Avi Pub. Co., Inc. 1970.
- Schwartz, D. P. New micromethods for isolating and characterizing lipid constituents. Agr. Sci. Rev. 8 (2&3) (1970) 41-50.
- Schwartz, D. P. Quantitative micro determination of alcohols as esters of pyruvic acid 2,6-dinitrophenylhydrazones. Anal. Biochem. 38 (1970) 148-157.
- Sinnamon, H. I. and Aceto, N. C. Vacuum foam-dried whole milk. Agr. Sci. Rev. 9(1) (1971) 35-42.
- Steinmaus, H., Elad, D. and Ben-Ishai, R. Ultraviolet light-induced purine modified DNA. Biochem. Biophys. Res. Commun. 40 (1970) 1021-1025.
- Thompson, M. P. Phenotyping milk proteins: A review. J. Dairy Sci. 53 (1970) 1341-1348.
- Timasheff, S. N. and Bernardi, G. Studies on acid deoxyribonuclease. VII. Conformation of three nucleases in solution. Arch. Biochem. Biophys. 141 (1970) 53.
- Webb, B. H. New dairy foods: Opportunities and obstacles. Food Eng. 42 (1970) 104-105, 107-108, 112-113.
- Weisenberg, R. C. and Timasheff, S. N. Aggregation of microtubule subunit protein. Effects of divalent cations, colchicine and vinblastine. Biochemistry 9 (1970) 4110.
- Zittle, C. A. Influence of phosphate and other factors on the rennin gel obtained with whole casein and with κ -casein in the presence of calcium salts. J. Dairy Sci. 53 (1970) 1013-1017.

FOREIGN MARKET DEVELOPMENT

- Berlin, E., Kliman, P. G., Anderson, B. A. and Pallansch, M. J. Calorimetric measurement of the heat of desorption of water vapor from amorphous and crystalline lactose. Thermochemica Acta 2 (1971) 143-152.

PROTECT FOOD SUPPLIES FROM HARMFUL MICROORGANISMS AND
NATURALLY OCCURRING TOXINS

Hargrove, R. E., McDonough, F. E. and Reamer, R. H. A selective medium and presumptive procedure for detection of Salmonella in dairy products. J. Milk Food Tech. 34 (1971) 6-11.

HUMAN NUTRITION

Edmondson, L. F., Douglas, F. W., Jr. and Avants, J. K. Enrichment of pasteurized whole milk with iron. Abstract. J. Dairy Sci. 53 (1970) 641.

Jones, S. B., Kalan, E. B., Jones, T. C. and Hazel, J. F. Ferripolyphosphate as a whey protein precipitant. Sixth Middle Atlantic Regional Mtg., ACS, Baltimore, Md., February 3-5, 1971, p. 47.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND
DISPOSAL OF WASTES

McDonough, F. E. Concentration and fractionation of whey by reverse osmosis. Proceedings Whey Utilization Conference, University of Maryland, College Park, Maryland. June 2-3, 1970, p. 36.

McDonough, F. E. and Hargrove, R. E. Sanitizing reverse osmosis equipment. J. Dairy Sci. 53 (1970) 645.

MEAT UTILIZATION

Problems and Objectives

Meat of improved stability, including enhanced retention of acceptable flavor and color characteristics, is needed so that more efficient processing and packaging in centralized locations with resultant economies is possible. Processed meat products, two-thirds of our pork and significant quantities of other meats, need better keeping quality relative to color and flavor retention in distribution channels. Processes need to be evaluated to prevent introduction of potential chemical health hazards into cured meat products. Technical advances are needed to enable the numerous small meat processors to manufacture better products. Contamination of meat with pathogenic microorganisms is a serious problem which must be eliminated through improved processing techniques.

Major objectives of the research are to develop and evaluate alternative ways to:

1. Provide procedures for the preparation of meat and meat products with initially desirable organoleptic and physical characteristics which are retained in storage.
2. Provide technology which can be applied to the problems of small-scale processors and improve the meat products of rural industry.
3. Improve meat processing methods so that the consumer can be assured of safety in meat and its products.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED MEAT PRODUCTS

Meat Products and Properties. Several new products have been developed from lamb meat in pre-cooked, frozen, boil-in-bag form. They have had good to excellent acceptance by laboratory and home user panels. The products are a convenience for the retail trade as well as for the hospital, restaurant and institutional purchaser. Included in the products are lamb curry, lamb shanks, lamb loaf, riblets and lamb cubes for shish-kabob. This research was conducted under contract at Pennsylvania State University, University Park, Pa.

At Tuskegee Institute, Tuskegee, Alabama, research under a contract was initiated to develop meat products with reduced water activity which would be more stable to microbial degradation and may offer possibilities

for the development of new type meat products. Glycerol was quite effective in reducing the water activity of ground beef, but none of the 17 gums studied proved effective.

Myosin is the most important of the meat fibrillar proteins. A more complete understanding of its activity as enzyme and as a polymer is necessary to solving the meat tenderness problem and many problems of binding in meat products. A preparative procedure for separation of myosin on hydroxyapatite was developed. An enzymically inactive fraction and an ATPase inhibitor were removed in this procedure and the presence of two components with ATPase activity was indicated. Serial use of cellulose phosphate and hydroxyapatite gives myosin of high purity and high enzymic activity. Conversion of an analytical procedure for myosin separation into a preparative method will permit accumulation of larger amounts of purified myosin for further investigation. The serial use of cellulose phosphate and hydroxyapatite to provide high purity myosin is an important technique for providing information on this essential enzyme and will assist in elucidating its role in muscle structure and meat properties. Further data on the structure were obtained by sulfonating myosin and separating it by electrophoresis.

In supporting research, an autoranging scale expander for spectrophotometer was built that increases precision and sensitivity of analogue data by a factor of five. This has been useful in monitoring effluents from chromatographic columns.

The volatile components of boiled beef were studied and 57 compounds identified in research conducted under a grant at Rutgers University, New Brunswick, N. J. Two interesting compounds associated with a fraction having meaty aroma are 2,4,5-trimethyl-3-oxazoline and 3,5-dimethyl-1,2,4-trithiolane. Implication of sulfur and nitrogen compounds in beef flavor is an important step in identifying key flavor components for potential modification. Flavor deterioration on storage is partially due to degradation of flavor components.

The disappearance of sodium nitrite (NO_2^-) in cured meat was studied at EMN with fractions extracted from fresh tissue. The extracts were found to metabolize NO_2^- and to develop color but at different rates. The reaction appeared to be due to reductants in the extracts which react in a manner similar to, but not directly involved in, reduction of nitrite. Metabolic intermediates that reduce nicotinamide adenine dinucleotide (NAD) enzymatically accelerate formation of nitric oxide myoglobin, but not to the extent that reductants do. Cured ham flavor, produced with NO_2^- , is separable from the basic meaty flavor, the pigment, and the fats and phospholipids of the meat. The flavor components are present in trace quantities and are difficult to separate and identify even with

gas chromatography and mass spectrometry. Studies on the fate of nitrite with meat extract fractions will furnish fundamental information on the curing process. Demonstration that mechanism of color formation may be different from that of reduction by other tissue components is an important factor and may be of use in modifying the cure process to produce a more desirable product.

Cured ham flavor, developed with nitrite (NO_2^-), is not associated with pigment or phospholipid fractions. Studies are continuing to improve gas chromatographic resolution, mass spectral analysis, and extraction procedures. Taste panel studies indicate that addition of NO_2^- to emulsion cure salts is necessary for development of frankfurter flavor. Frankfurter flavor is also associated with smoke. In the absence of smoke, or in lightly smoked franks, the absence of NO_2^- is readily detected. Heavy smoke modifies flavor so absence of NO_2^- is more difficult to detect. Studies are continuing to determine levels of NO_2^- needed for flavor. Information on the fate of the nitrite used in the cure, both from the chemistry of the reduction process and from the aspect of the flavor compounds formed, will be of great value in determining future work on the processing of cured meat products.

Meat Processing. The effect of calcium on the stability of frankfurter emulsion was investigated using a system with marginal emulsion stability. The emulsions were comminuted to 75°F with samples removed at 55°, 65° and 75°F. The samples were stuffed, smoked and cooked to an internal temperature of 160°F. They were then checked for release of fat. Even under these conditions there was no relationship between calcium content and stability. Contrary to the industry claim for greater stability of emulsion made with calcium reduced nonfat dry milk, addition of this product or nonfat dry milk had little effect. Sodium nitrite in frankfurter cure is necessary for development of color, and spectrophotometric methods showed increasing color with greater nitrite concentrations. However, visually, only the difference between no nitrite and color production with nitrite could be detected.

Some metals are involved in the oxidation of meat lipids. Addition of fatty acids to model systems containing lard increased the prooxidant activity of ferrous ions but inhibited the action of cobaltous ions. The action of the antioxidant α -tocopherol was decreased by fatty acids in the presence of ferrous ions and increased by cobaltous ions. Previous work showed that some bacteria produced antioxidant compounds. Other types of microorganisms have now been studied and it was found that little antioxidant activity was produced by the limited number of streptomyces studied, only Candida lipolytica showed any activity among the yeasts investigated, and the fungi had some activity. The anti-oxidative effect produced by Aspergillus niger material is being isolated and

studied chemically. A procedure has been developed for isolating α -dicarbonyls, and the α -dicarbonyl content of a number of triglycerides and lard were determined. Lard contained approximately 30 μ moles/mole (assuming a MW of 1000 for lard).

Ground beef sterilized by Cobalt-60 irradiation was found useful for studying microbial changes in meat fat. When stored for 2-3 weeks at 37°C, marked changes occur in the monocarbonyl fraction of the fat. This is interesting from the standpoint of the stability of irradiated meat products.

The contribution of brine microorganisms to the meat curing process is being investigated under a PL-480 grant at Yugoslav Institute of Meat Technology, Belgrade. For the isolation and propagation of salt-tolerant bacteria, it was established that the media should have a salt concentration of at least 2.5%. Media with higher than 2.5% salt concentration were used only for isolation of halophilic and related bacteria. In isolation methods employing culture dilution, best results to date were obtained by using brine passed through a Seitz filter as the diluent.

At EMN, a rapid method was developed for the analysis of fats in meat products. The procedure reduces by 80% the time required for the official AOAC method with a 97-100% recovery of fat in ground beef, frankfurters, and pork sausage. Methods for rapid analysis of moisture in meat products were studied and an inexpensive procedure requiring less than one hour is being recommended for consideration in routine quality control. A moisture recovery of better than 95% is obtained by the rapid method. The work on the methods of analysis is important for the development of simple, inexpensive procedures that can be used by small scale meat processors with limited experience in chemical analysis.

INSURE FOOD PRODUCTS FREE OF TOXIC RESIDUES

Nitrosamines. A quantitative procedure sensitive to 25 μ g/kg was developed for the determination of N-nitrosodimethylamine (DMNA). A number of ham samples, domestic and foreign, were examined and found to contain less than 1 μ g/kg of apparent DMNA. Formation of DMNA in model systems containing dimethylamine and NO_2^- is optimal at pH 3.5. At pH 2.0 and pH 5.6 (the average values for gastric juice and meat respectively) essentially no DMNA is formed. Under conditions simulating meat cooking, DMNA is formed from the reaction of betaine, a naturally occurring quaternary ammonium compound, and nitrite. The formation of DMNA from quaternary ammonium compounds has not been reported previously. The work with betaine involved a study of the mechanism of reaction, the identification of intermediate compounds and the conditions for formation.

PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISMS

Control Procedures. Development of a method for utilizing radiation killed cells of Pediococcus cerevisiae for acid production in meat products as a means of inhibiting undesirable bacteria has continued in research under a grant at the University of California, Davis, California. The ability of irradiated cells to produce acid from glucose was not impaired by freezing and storage for periods up to 10 months.

Studies at Veterinary Research Institute, Pulawy, Poland under a PL-480 grant complement the investigation of indigenous microflora at Davis, California. Inhibitory effects have been observed for certain microorganisms but these were also attributable to pH reductions due to acid production of the competing species.

Under PL-480 sponsored research at University of Veterinary Medicine, Vienna, Austria, lauryl sulfate was found to be bacteriostatic for both spores and vegetative cells in heat treated meat-fat emulsions (25% beef, 33% lard, 9% milk protein and 33% water). In the presence of added nitrite the lauryl sulfate was effective at the 0.005% level. When the beef content of the emulsion was increased to 45%, however, no effect was obtained regardless of concentration of lauryl sulfate or additional use of polyphosphates. Mixtures of lysozyme polyphosphate and an erythrocyte extract were also bacteriostatic under experimental conditions. The activity of the erythrocyte extract was reduced or neutralized in material destined for sausage making, apparently due to the extract being absorbed to muscle protein; there is no affinity for connective tissue protein.

Toxigenicity of staphylococci is not readily transmissible by the phospholipid fractions of high enterotoxin-producing strains. This material had no effect on the toxin production of strains normally forming low levels of enterotoxin.

Detection Procedures. Studies at EMN on detection and identification of salmonellae in meat and bone meals showed that smaller samples (30 gram) were more efficient for isolating different serotypes; two days' incubation enhanced the percentage of isolates that could be identified as salmonellae although the number of positive meat and bone meals was the same; tetrathionate enrichment favored the isolation of one somatic group of Salmonella while selenite-cystine favored the isolation of several other types. The picking of isolated presumptive colonies into lysine-iron slants was shown to be feasible and eliminated the need for an intermediary step of using triple sugar iron medium. A total of 1724 salmonellae isolates were serotyped and several apparently lactose-positive salmonellae were isolated from meat and bone meal.

The International Committee on Microbiological Specifications has made considerable progress in promoting collaborative studies on evaluating methods for recognizing salmonellae in food products. To obtain 95% confidence limit for the absence of the organism 60,25-gram food samples would have to be analyzed. In an effort to reduce this tremendous analytical load, a study was carried out which indicates analysis of three 500 gram samples will yield a level of sensitivity equivalent to that of the smaller samples. A protocol has been developed for a collaborative study on methodology of evaluating coliforms. Incorporation of several diazines into trypticase soy broth resulted in a medium selective for the growth of pseudomonads. Media and analytical procedures were developed for the qualitative and quantitative estimation of pyocyanine, phenazines, pyrrobrubin and two fluorescent pigments produced by Pseudomonas aeruginosa.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED MEAT PRODUCTS

- Ackerman, S. A., Swift, C. E., Carroll, R. J. and Townsend, W. E.
Effects of types of fat and of rates and temperatures of comminution
on dispersion of lipids in frankfurters. J. Food Sci. 36 (1971)
266-269.
- Cho, I. C. and Bratzler, L. J. Effect of sodium nitrate on flavor of
cured pork. J. Food Sci. 35 (1970) 668-670.
- Cohen, E. H. Rapid technique for solvent removal in the determination
of fat. J. Assoc. Offic. Anal. Chemists 54 (1971) 212-214.
- Connelly, J. A., Mozersky, S. M. and Pessen, H. An autoranging scale
expander. Rev. Sci. Instr. 42 (1971) 773-776.
- Cormier, A., Wellington, G. H. and Sherbon, J. W. Epimysial connective
tissue polysaccharides of bovine semimembranosus muscle and alterations
in their type with age and sex differences. J. Food Sci. 36 (1971)
199-205.
- Doerr, R. C. and Fiddler, W. Partition ratios of some wood smoke phenols
in two oil:water systems. J. Agr. Food Chem. 18 (1970) 937-939.
- Fiddler, W., Wasserman, A. E. and Doerr, R. C. A "Smoke" flavor fraction
of a liquid smoke solution. J. Agr. Food Chem. 18 (1970) 934-936.
- Macy, R. L., Jr., Naumann, H. D. and Bailey, M. E. Water-soluble flavor
and odor precursors of meat. 3. Changes in nucleotides, total nucleo-
sides and bases of beef, pork and lamb during heating. J. Food Sci.
35 (1970) 78-80.
- Macy, R. L., Jr., Naumann, H. D. and Bailey, M. E. Water-soluble flavor
and odor precursors of meat. 4. Influence of cooking on nucleosides
and bases of beef steaks and roasts and their relationship to flavor,
aroma and juiciness. J. Food Sci. 35 (1970) 81-83.
- Piotrowski, E. G., Zaika, L. L. and Wasserman, A. E. Studies on aroma
of cured ham. J. Food Sci. 35 (1970) 321-325.
- Randall, C. J. and Bratzler, L. J. Effect of smoking process on solu-
bility and electrophoretic behavior of meat proteins. J. Food Sci. 35
(1970) 245-247.

- Randall, C. J. and Bratzler, L. J. Changes in various protein properties of pork muscle during the smoking process. J. Food Sci. 35 (1970) 248-249.
- Randall, C. J. and Bratzler, L. J. Effect of smoke upon acid phosphatase activity of smoked meat. J. Food Sci. 35 (1970) 250.
- Townsend, W. E., Ackerman, S. A., Witnauer, L. P., Palm, W. E. and Swift, C. E. Effects of types and levels of fat and rates and temperatures of comminution on the processing and characteristics of frankfurters. J. Food Sci. 36 (1971) 261-265.
- Wasserman, A. E. and Spinelli, A. M. Sugar-amino acid interaction in the diffusate of water extract of beef and model systems. J. Food Sci. 35 (1970) 328-332.
- Zaika, L. L. Column chromatography on polystyrene resin using aqueous systems. J. Chromatog. 49 (1970) 222-229.

PROTECT FOOD AND FEED SUPPLIES FROM HARMFUL MICROORGANISMS

- Enkiri, N. K. and Alford, J. A. Relationship of the frequency of isolation of salmonellae to their resistance to drying and freezing. Appl. Microbiol. 21 (1971) 381-382.
- Palumbo, S. A. and Alford, J. A. Inhibitory action of tetrathionate enrichment broth. Appl. Microbiol. 20 (1970) 970-976.
- Rose, M. J., Enkiri, N. K. and Alford, J. A. Growth and survival of enterobacteria in selenite-cystine broth containing thiosulfate. J. Food Sci. 36 (1971) 590-593.

ANIMAL FATS AND OILS UTILIZATION

Problems and Objectives

Finding outlets for inedible fats has been a problem ever since synthetic detergents began to replace soap. The problem has been complicated further by the increase of fat production associated with the continuing increase in meat consumption. Previous EMN research activities have been effective in providing markets for over one-fourth of the 4.7 billion pounds of inedible fat that is produced per year. In order to maintain current markets and provide for new and economical ones for this byproduct of the meat industry a strong and progressive research program is necessary. A promising area that is accounting for the use of an increasing percentage of the inedible fat production is that of chemical derivatives. Continued research is needed to provide the chemical industry with new products and processes. In the area of pollution, tallow-based detergents and lime soap dispersing agents, such as sulfated alkanolamides, are easily biodegradable under both aerobic and anaerobic conditions and hence do not persist in the environment. Although the role of phosphates in eutrophication requires clarification, the development of tallow-based phosphate-free detergent formulations is warranted. At the same time, basic and exploratory research on the organic and physical chemistry of fats and fatty acids is needed in order to maintain a continuing supply of fundamental knowledge; to establish new principles and concepts; and to discover new reactions and processes for future development.

Major objectives of the research are to develop:

1. Fundamental knowledge of the composition, structure, and physical and chemical properties of inedible fat and its components.
2. New and improved techniques for separating and characterizing the components and derivatives of fat.
3. New chemical intermediates.
4. New and improved industrial products--particularly for use as biodegradable detergents, plastic foams and coatings and lubricant components and additives.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

Polymers. Fire-resistant, low-density rigid urethane foams were prepared from hypohalogenated lard, tallow, soybean oil or monoolein, by reaction

with a polyarylene polyisocyanate. Hypobrominated lard and tallow, freed from unreacted saturates by solvent precipitation, gave enriched polyols generating foams with improved properties. Hypochlorination of unsaturated fats by direct addition of hypochlorous acid, usually performed in aqueous suspension, was made more efficient by a solution process. Preliminary cost estimates for the commercial-scale production of hypohalogenated tallow from epoxidized tallow indicate that the process is potentially profitable.

Copolymers of octadecyl acrylate with acrylonitrile or methyl methacrylate showed promise in screening tests as viscosity-index improves for lubricating oils. Allyl stearate, at moderate concentrations, appears comparable to vinyl stearate as an internal plasticizer for co- or terpolymers with vinyl chloride. Monovinyl carboxystearate, made by carbon monoxide reaction with oleic acid followed by vinylation, was difficult to purify and to polymerize to useful products. Chain-transfer constants for a series of monomers in methyl oleate as solvent showed that comonomers carrying the non-crystallizing oleyl unit in the side chain could be copolymerized with relatively reactive monomers without excessively depressing degree of polymerization.

Isopropenyl Esters. Vapor-liquid equilibrium data for the acetone-acetic anhydride-isopropenyl acetate (IPA) system have been obtained, and are being correlated. These data are required in the separation stage of the IPA process. Two contaminants in the propyne-stearic acid process have been identified as 1-propenyl stearate and vinyl stearate. By gas chromatography analysis a third, but unknown, byproduct has been found. In a newly discovered synthetic reaction, isopropenyl stearate reacts under forcing conditions with methyl acetylene to yield an unusual conjugated acetylenic enol, i.e., 4-oxo-2-heneicosyne.

Engineering studies are underway on the reaction of propyne and stearic acid to form isopropenyl stearate (IPS). A bench-scale simulated continuous process for producing IPS from the reaction of isopropenyl acetate and stearic acid is in the design and fabrication stage. Recovery schemes for both processes have been developed, and testing is underway. Flow sheets for both processes have been prepared. A 200-lb. supply of crude isopropenyl stearate was purified in an industrial evaporator for future commercial evaluation.

α -Anion Procedure. The new metalation (or α -anion) reaction for allowing direct substitution of fatty acids at the α -position has been extended to include reaction with formaldehyde to form α -alkylhydracrylic acids. A facile dehydration of these compounds affords α -alkyl acrylic acids. These are the first high yield syntheses devised for long-chain α -alkyl hydracrylic and acrylic acids. The α -metalation reaction was also used

to study the isomerization of 2-olefinic to 3-olefinic acids and to prepare cis- and trans-isomers and α -substituted derivatives of the latter. NMR study of the addition of thiocyanogen to model olefins showed that the thiocyanate adducts can be cyclized to 2-imino-1,3-thiolane salts. Initial homologous members of the thiolane series are reported in the literature to be pesticides and herbicides. Since higher, fatty homologs were not successfully prepared by the traditional cyclization method, a new method with methanesulfonic acid as solvent was developed for their synthesis.

Lubricants. Efforts to prepare lubricants and lubricant components from animal fats have been continuing. Stearic acid has been isomerized to liquid C₁₈ acids via two different approaches. In the first of these, heptadecenes, obtained by the action of palladium chloride on stearoyl chloride, were converted to non-linear (branched chain), saturated C₁₈ acids by reaction with formic and sulfuric acids. In the second approach, the heptadecenes were converted to non-linear (saturated) C₁₈ aldehydes, under pressure, in the presence of carbon monoxide, hydrogen and a rhodium-triphenylphosphine catalyst. These aldehydes were then readily oxidized with potassium permanganate to the corresponding non-linear C₁₈ acids. Such C₁₈ saturated liquid acids have potential utility in a number of commercial applications.

In attempts to prepare a sperm oil substitute, various glycerides and/or mixtures of glycerides, essentially from animal sources, have been converted to their corresponding wax esters. The reaction sequence includes reduction and saponification of the glycerides to alcohols and to acids respectively, followed by esterification to wax esters. Glycerides have been chosen so as to yield wax esters with properties (e.g. iodine value, chain length, oxidative stability) similar to those of natural sperm oil.

Studies have continued on the synthesis of fatty aziridines and their utilization as lubricant additives. In efforts to synthesize such compounds less expensively, the use of three new olefinic reagents have been explored. These, N,N-dichlorobenzenesulfonamide, nitrosyl chloride and iodine azide, have been reacted with model olefinic compounds. Subsequent reactions have yielded N-sulfonylaziridines, and a mixture of azirenes and aziridines from the dichlorobenzenesulfonamido- and iodine azide addition products, respectively. The conversion of the nitrosyl chloride addition products to cyclic N derivatives has not been accomplished. The direct methylation of sodium cis-9,10-epiminooctadecanoate (preserving the aziridine ring) has been achieved under a variety of conditions, the optimum utilizing boron trifluoride and methanol. A number of soaps of cis-9,10-epiminooctadecanoic acid have been prepared and are being tested as possible grease formers. In addition, greases have been formulated from soaps of cyanoethylated fatty acids.

Supporting Research. Thermally stable liquid phases for gas liquid chromatography (GLC) were studied under a PL-480 grant at Polytechnic University, Gdansk, Poland. Ethylene glycol succinate type polyester with a "linear" structure, stabilized with 1% phosphoric acid, or polyester of N-methyldiethanolamine with succinic acid is recommended for the GLC separation of saturated and unsaturated fatty acids. The studies confirmed that the manner of preparation of the polyester has an effect on thermostability.

The study of enzyme systems and the in vivo synthesis of epoxyoleic acid in Vernonia anthelmintica is in progress under a PL-480 grant at University of Calcutta, Calcutta, India. A micromethod selective for long chain compounds containing primary or secondary hydroxyl functions has been developed, but the method appears to be limited to determination of monohydroxy compounds.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

Biodegradable Detergents. As little as 3-10% (basis soap) tallow-derived α -sulfoesters, sulfated alkanolamides, ether alcohol sulfates and Igepons (2-sulfo-ethyl esters or N-methyltaurides of tallow acids) added to tallow soap were found to maintain lime soap in suspension in water having hardness in excess of 300 ppm CaCO_3 . These lime soap dispersing agents (LSDA) were effective in maintaining the dispersion upon further hard water dilution simulating rinsing.

Increasing the level of LSDA to 10-20% improved the detergency of tallow soap in hard water. Further improvements in detergency were obtained by the addition of small amounts of detergent builders such as sodium silicate, sodium tripolyphosphate, sodium citrate, sodium oxydiacetate or sodium nitrilotriacetate. A study of such ternary systems showed that optimization of detergency, as determined by reflectance measurements, occurred at an approximate composition of 80% soap, 10% lime soap dispersing agent and 10% builder.

Beneficial effects of phosphates were not readily evident in single washing of standard soiled cotton swatches. When cotton swatches were repeatedly soiled with vacuum cleaner sweepings and washed through six cycles, phosphate built detergents gave significantly cleaner swatches than the same detergent built with a mixture of silicates, bicarbonates and sulfates having the same pH. In addition, this evaluation method confirmed that a ratio of 80% soap-10% lime soap dispersing agent-10% builder gave optimum detergency for that three component system. Glycol, glycerol and pentaerythritol esters of α -sulfonated fatty acids were found to possess solution properties, lime soap dispersing ability and detergency properties similar to those of sucrose and hexitol esters of α -sulfonated fatty acids.

An improved analytical technique for measuring the aerobic as well as microaerophilic biodegradation of anionic surfactants was developed. Sulfated alkanolamides were found to be biodegraded in aerobic, microaerophilic and anaerobic systems but were most rapidly biodegraded under aerobic conditions. No detergent was detected in the effluents after 20 ppm solutions of sulfated alkanolamides were percolated through a silt loam soil.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

- Barford, R. A., Rothbart, H. L. and Bertsch, R. J. Preparative fractionation by frontal countercurrent distribution. *Sepn. Sci.* 6 (1971) 175-185.
- Chakrabarty, M. M., Bhattacharyya, D. and Kundu, M. K. A simple photometric method for microdetermination of fatty acids in lipids. *J. Am. Oil Chem. Soc.* 46 (1969) 473-475.
- Chakrabarty, M. M., Bhattacharyya, D. and Kundu, M. K. A micro-titrimetric method for the determination of the oxirane functional group. *Analyst* 95 (1970) 85-87.
- Foglia, T. A., Gregory, L. M. and Maerker, G. Stereochemistry of the isomerization of N-acyl-2,3-disubstituted aziridines to Δ^2 -oxazolines. *J. Org. Chem.* 35 (1970) 3779-3785.
- Foglia, T. A., Maerker, G. and Smith, G. R. Preparation of epimino-stearates. Comparison of methods. *J. Am. Oil Chem. Soc.* 47 (1970) 384-388.
- Herb, S. F. and Martin, V. G. How good are analyses of oils by GLC? *J. Am. Oil Chem. Soc.* 47 (1970) 415-421.
- Jordan, E. F., Jr., Bennett, R., Shuman, A. C. and Wrigley, A. N. Reactivity ratios and copolymerization parameters for copolymers incorporating n-octadecyl acrylate and N,n-octadecylacrylamide. *J. Polm. Sci. A-1* (1970) 3113-3121.
- Maerker, G., Haeberer, E. T., Donahue, E. T. and Foglia, T. A. Reaction of cis-2,3-dioctylaziridine with carboxylic acids. *J. Hetero. Chem.* 7 (1970) 563-567.
- O'Connor, R. T. and Herb, S. F. Specifications of fatty acid composition for identification of fats and oils by gas liquid chromatography. *J. Am. Oil Chem. Soc.* 47 (1970) 186A, 195A, 197A.
- Osman, S., Dooley, C. J., Foglia, T. A. and Gregory, L. M. The characterization of isomeric oxazoline derivatives by mass spectrometry. *Org. Mass Spect.* 4 (1970) 139-145 (Supplementary 1970).

Rothman, E. S. and Moore, G. G. Enol esters. XII. C-Acylation with enol esters. J. Org. Chem. 35 (1970) 2351-2353.

Rothman, E. S. and Moore, G. G. A simple synthesis of the enol sterate of 4-oxo-2-heneicosyne. Tetrahedron Letters 16 (1971) 1065-1066.

Scholnick, F., Saggese, E. J., Wrigley, A. N. and Riser, G. R. Urethane foams from animal fats V. Flame resistant foams from hypohalogenated glycerides. J. Am. Oil Chem. Soc. 47 (1970) 180-182.

Turner, D. L., Silver, M. J., Baczynski, E., Holburn, R. R., Herb, S. F. and Luddy, F. E. The synthesis of phosphatidylethanolamine and phosphatidylserine containing acetylenic or cyclopropane fatty acids and the activity of these phosphatides in blood coagulation. Lipids 5 (1970) 650-657.

Rothman, E. S. Process for preparation of hexadecylketene. U. S. Patent 3,535,383, October 20, 1970.

Rothman, E. S. and Serota, S. Preparation of stearoyl fluoride. U. S. Patent 3,549,675, December 22, 1970.

Silbert, L. S. Process for the preparation of alkyl, perfluoroalkyl and aryl iodides. U. S. Patent 3,531,535, September 29, 1970.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

Bistline, R. G., Jr. and Stirton, A. J. Reduction of phosphate builder in tallow-based detergent formulations. J. Am. Oil Chem. Soc. 48 (1971) 74-76.

Maurer, E. W., Cordon, T. C. and Stirton, A. J. Microaerophilic biodegradation of tallow-based anionic detergents in river water. J. Am. Oil Chem. Soc. 48 (1971) 163-165.

Smith, F. D. and Stirton, A. J. Phenylstearic acid and related compounds. Isolation of 17-, 16-, 15- and 13-phenyl isomers. J. Am. Oil Chem. Soc. 48 (1971) 160-162.

Weil, J. K., Parris, N. and Stirton, A. J. Sulfated diglycolamides. J. Am. Oil Chem. Soc. 48 (1971) 35-37.

HIDES AND LEATHER UTILIZATION

Problems and Objectives

Finding profitable outlets for hides has been a continuing problem since synthetic substitute leather-like materials began to replace leather. The problem has been further compounded by the increase of hide production as a result of the continuing increase in the slaughter of cattle. In order to maintain current markets and provide for new and economical ones for this important product of the cattle industry, which accounts for 6-10% of the live weight of the cattle, a strong research program is necessary. Areas in which the current research program seeks to improve the market for hides are in the development of leathers with new and unique properties and in the development of food uses. Another problem to be solved if the leather industry is to survive is the control of pollution resulting from the processing and tanning of hides. Areas of needed research include processes to recover protein and fat from fleshings, trimmings and hair; preservation of hides without the use of salt, and elimination or reduction of content of soluble and insoluble solids in tannery effluents. At the same time a strong basic and exploratory research program is needed in order to maintain a continuing supply of fundamental knowledge regarding the physical and organic chemistry of collagen which is necessary for future developments.

Major objectives of the research are to develop:

1. Fundamental knowledge of composition, structure, and physical and chemical properties of hides and their components.
2. Procedures for attaining high quality raw stock.
3. Pollution control during processing and tanning of hides.
4. New and improved techniques for processing hides into leathers.
5. Chemical modifications of hides that result in the development of leathers with unique and desired characteristics.
6. Edible uses for the collagen of cattle hides.

Progress - USDA and Cooperative Program

NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

Improved Products. Deerskin leather tanned with glutaraldehyde or with this aldehyde in combination with basic chromium sulfate (chrome) can be

penetrated more easily with a needle than leather tanned with chrome alone. These results suggest that leather tanned with glutaraldehyde is easier to sew than leather tanned with chrome alone. Glutaraldehyde-tanned Australian opossum skins, proved durable by trials in textile mills, are in commercial production. The fur is used in the loom shuttles to prevent knotting by providing proper tension on the fiber. A comparison of the physical and chemical properties of cattlehide and horsehide garment leathers indicates that the former is suitable for policemen's coats when the leather meets the specifications for strength. This would allow the use of readily available domestic hides instead of the imported horsehides, which are in short supply.

Glutaraldehyde imparts to shearlings perspiration resistance and resistance to washing in hot soap solution. However, these products are highly hydrophilic which makes drying more difficult. Treatment of washable shearlings with several chromium complex type water repellents drastically reduces the time required for drying after laundering. They can be air-dried in a reasonable time (6 hours) by simply hanging them on a line at ambient conditions. Thus machine drying is not necessary. The elimination of the tumbling, as in a dryer, reduces clumping of the wool and helps preserve the fluffy appearance of the shearlings which is desirable for rugs, wall displays, etc. The chrome complexes successful in this application were Scotchgard (a fluorochemical), Quilon (stearate chromic chloride) and Pentel 52 (a fluorochemical). These are available commercially and are used on a limited scale for waterproofing leather. The effects of these materials are evident through at least four wash-dry cycles. Washing shearling bedpads in a laundry product containing sodium perborate will shorten their useful life.

Research was begun to evaluate some flame retardant chemicals as treatments for glove leather so as to develop flyer's gloves to meet the need of the military. Impregnation with halogenated phosphate esters gave very promising results. Tris(dibromopropyl) and tris(chlorobromopropyl) phosphate were most effective, reducing the after-flame and after-glow of the treated glove leather to almost zero. The impregnated leathers also appeared to retain the stretch (or run) desirable for glove leather.

Preliminary experiments using chrome-tanned sheepskins as the substrate and ethyl or butyl acrylates as the monomer indicate that graft polymerization could be a practical way of manufacturing leathers with interesting new and improved properties. Grafted poly (butyl acrylate) acts as a lubricant and also gives the leather a high degree of area stability.

Deriphat 151C (a N-fatty amino acid derivative) has been shown previously to be useful as a leather fiber lubricant with special promise of application on garment leather. This material, unlike those lubricants now used

commercially, is quite resistant to removal by common drycleaning solvents such as Stoddard solvent, perchloroethylene and Valclene. The low solubility of Deriphat 151C in chlorinated solvents precluded the use of the ALCA standard method of analysis using chloroform. A satisfactory method of analysis using 95% ethyl alcohol as a solvent was developed. Experiments on carefully prepared suede leather samples, with the aid of this new technique, have shown that essentially no Deriphat 151C is lost to drycleaning solvents including those used in "coin-op" machines. Commercial interest has developed in this work and license applications to operate under our U. S. Patent No. 3,300,338 have now been received from several tanners.

Tanning Investigations. In experimental studies of the chrome tanning of cowhide, an empirical mathematical relationship was developed which accurately relates chrome fixation at equilibrium to chrome concentration and float ratio. A computer program which will describe the tanning rate as a function of all the pertinent experimental variables is being developed and tested.

Laboratory tanning of sole leather at Central Leather Research Institute, Madras, India, under a PL-480 grant has been adapted to pilot plant scale operation. Tanning of buffalo hides is commenced in myrobalan (pyrogallol type tannins) and after the tannins have struck through the cross section of the hide, tanning is completed with wattle or other catechol tannins.

In supporting research, an electrodialysis cell, into which a hide piece can be inserted without dismantling the cell, was designed and tested at EMN. With adequate cooling and flushing of the electrode compartments, the content of anions and cations present in a hide piece can be determined within one percent. This will aid studies to develop electrical methods to determine hide condition during processing.

Investigation of the chemical composition of cattle hair which has been exposed to various unhairing treatments indicates that considerable changes have taken place. Considerable amounts of the cystinyl residues are converted to lanthionyl residues, some of the lysinyl residues interact with intermediates formed during the treatment to yield lysinoalanyl residues and dimethyl amine, if used in unhairing, also interacts to form β -dimethylaminoalanyl residues. The intermediate which is presumably formed (from cystinyl and seryl residues) and involved in the formation of the above less common amino acids is the dehydroalanyl (α -aminoacrylyl) residue. These findings indicate that the reaction involved is a base-catalyzed β -elimination reaction from cystinyl and seryl residues and not a nucleophilic substitution reaction as previously proposed.

Fundamental aspects of tanning are being studied under a PL-480 grant at Hebrew University of Jerusalem, Israel. Kinetic measurements involving polylysine (PL) and glutaraldehyde (GA) revealed the existence of at least two steps in the proton liberation at a constant pH, particularly in the pH 8-10 range. The reaction at pH 10 was found to be much faster than previously recorded. Hydrodynamic measurements indicate that at relatively low concentrations of GA (1:4 ratio with PL) the interaction leads essentially to an intramolecularly crosslinking of the two structures. At a GA:PL residue ratio of 1:1, intermolecular crosslinking also occurs. Some similarities in kinetic behavior were observed when collagen was used in the system instead of the model substance PL.

Hide Defects. Histological study of hide biopsies from 60 twin heifers in a comparative feeding test gave strong presumptive evidence, at one year of age, that the vertical fiber defect is genetically transmitted among Herefords but is absent in Holsteins. Additional samples at two years of age are being processed to establish the accuracy of these findings, as well as the relationship between dietary level and internal fat in the hides. A penetrometer device for nondestructive detection of vertical fiber in cured hides gave poor correlation with leather strength in a large tannery test. However, when applied to leather, the device showed excellent correlation with the standard ball burst test and should be useful as a new quality control test. The instrument is being modified to improve its structural specificity.

A cooperative (ASRD) study on freezebranding has demonstrated that brand legibility, tissue effects, and leather damage can be correlated with variations in branding conditions. A second cooperative (VSRD) survey of hides in process showed a very high incidence of Demodectic mange in beef cattle, with a lower degree of severity in steers compared with mixed steers and heifers.

NEW ANIMAL FOOD PRODUCTS

Collagen Dispersions for Food Uses. Additional technical data necessary for estimating the cost of producing high solids collagen dispersions were collected by determining the power output over a wide range of operating conditions in the different comminuting machines which comprise the processing line. A high speed pump has recently been added to take better advantage of the high capacity of the grinding equipment. Force feeding the Urschel Comitrol, the third out of four comminuting machines in the processing line, eliminates the necessity of cooling when grinding neutral hide at about 25% solids content through a 0.06" cutting head. Force feeding the disc mill (the last of the grinding machines) requires cooling and is still under investigation. The microcut (0.015") Comitrol head has been operated with limited success using precut (0.06") material diluted

with an equal weight of water. A bacteriological contamination problem has been identified and solved by a novel pH adjustment technique. Assuming an annual output of 4 million pounds of product ground through the 0.06" cutting head of the Urschel Comitrol at neutral pH, a preliminary cost estimate indicates that the factory selling price should be about 32 cents per lb., including 20 cents per lb. for the raw material.

Viscosity measurements have been conducted on dispersions of collagen made under various conditions of acidity and temperature. Neutral and mildly acid warm dispersions of collagen were found to be less viscous than the corresponding cold dispersions. On cooling these dispersions produced gels that are considerably stronger than gelatin. They may be made at collagen concentrations up to 35%. Gels and films made from warm dispersions show resistance to boiling water. They become tender but do not dissolve or disintegrate. This is desirable in food use. Rat-feeding tests at WMN showed that an extruded mixture of 25% collagen and 75% soy flour had a Protein Efficiency Ratio of 1.4, when casein is set at 2.5. Collagen is an excellent binder for fish and shrimp feeds.

In supporting research, electric birefringence measurements show that the presence of an electric field promotes aggregation of dissolved collagen and this can be carried to the point where a tough transparent collagen film forms on the cathode. Extrapolation of decay data to zero and to infinite pulse widths shows that dissolved acid soluble collagen is a mixture of monomeric and dimeric species. The permanent dipole moment of dissolved collagen is constant under all conditions of concentration, pH, ionic strength, and temperature. The induced dipole moment varies with all of these parameters and also with time duration of the electric field. Collagen fractions separated via ultracentrifugation show identical electric birefringence patterns indicating that both dimer and monomer have the same sedimentation constant. This is possible only if aggregation to form the dimer is end-to-end.

In research under a contract at Northwestern University, Evanston, Illinois, viscosity studies of solutions of acid soluble collagen showed it to be present as a mixture of monomer and dimer. Pronase or sonication converts this to pure monomer. Chain sequences of collagen rich in amino acid residues are more stable than other regions. This shows that hydrogen bond strength is a function of degree of proton acceptance. Interchain cross-links enhance renaturation rates. In vivo monomeric collagen aggregates to form structural units of four or five molecules which are staggered axially by successive 200 Å displacements from the carboxy terminal end. Fibrils of indefinite length are then created by stacking the structural units endwise.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

As an approach to pollution abatement from the hide processing and curing operations, a number of germicidal products were screened for their effectiveness in the short term preservation of hides without the use of salt. The following combinations preserved specimens of fresh hides for up to two weeks: (a) 2% sodium bisulfite; (b) 1% sodium bisulfite plus 2% sodium bisulfate; and (c) 0.2% Dextraset UN in a weakly acid medium. Because of their low cost and relative innocuous nature, these compounds deserve further consideration and will be evaluated under practical conditions.

Very efficient and economical methods for the preparation of amino acid esters and for their conversion to the acetylated derivatives have been developed. The production of volatile amino acid derivatives by an economical process should enable the preparation of amino acids from waste proteins such as hide scraps which are a disposal problem for tanneries.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED NON-FOOD ANIMAL PRODUCTS

- Brownlowe, M. A., Cohen, F. R. and Happich, W. F. New washable woolskins. Am. J. Nursing 70 (1970) 2368-2370.
- DellaMonica, E. S. and McDowell, P. E. Atomic absorption spectroscopy versus alkaline fusion method for determining chrome in leather. J. Am. Leather Chemists Assoc. 66 (1971) 21-30.
- Everett, A. L. and Bitcover, E. H. The common origin of the seedy and ticky defects of sheepskin. J. Am. Leather Chemists Assoc. 65 (1970) 402-409.
- Everett, A. L., Hannigan, M. V., Bitcover, E.H., Windus, W. and Naghski, J. Hereditary influence on vertical fiber structure and strength variation in side upper leather. J. Am. Leather Chemists Assoc. 66 (1971) 161-177.
- Everett, A. L., Roberts, I. H. and Naghski, J. Reduction in leather value and yields of meat and wool from sheep infested with keds. J. Am. Leather Chemists Assoc. 66 (1971) 118-132.
- Fein, M. L., Viola, S. J. and Filachione, E. M. Colorfast dyes for washable and drycleanable leather. J. Am. Leather Chemists Assoc. 65 (1970) 584-592.
- Hannigan, M. V., Windus, W. and Naghski, J. Glutaraldehyde treatment of insole leather. J. Am. Leather Chemists Assoc. 65 (1970) 451-456.
- Happich, W. F. and Windus, W. Washable shearling bedpads deteriorated by perborate detergents. Hosp. Bureau Research News 18 (6) (1971) 1.
- Nayudamma, Y., Hemalatha, R. and Joseph, K. T. Investigation on the interaction of dialdehydes and polyaldehydes on collagen. J. Am. Leather Chemists Assoc. 64 (1969) 444-452.
- Rao, K. P., Joseph, K. T. and Nayudamma, Y. Propfpolymerisation von vinylmonomeren auf kollagen unter mitwirkung von cer IV-Ionen. Das Leder 19 (1968) 77-83.
- Rao, K. P., Joseph, K. T. and Nayudamma, Y. Grafting of vinyl monomers onto modified collagen by ceric ion - Studies on grafting site. Leather Science 16 (1969) 401-408.

Volpin, D. and Veis, A. Isolation and characterization of the cyanogen bromide peptides from the $\alpha 1$ and $\alpha 2$ chains of acid soluble bovine skin collagen. Biochemistry 10 (1971) 1751.

Wilkoff, L. J., Dixon, G. J., Westbrook, L. and Happich, W. F. Potentially infectious agents associated with shearling bedpads. II. Effect of laundering with detergent-disinfectant combinations on Staphylococcus aureus and Pseudomonas aeruginosa. Appl. Microbiol. 21 (4) (1971) 647-652.

NEW ANIMAL FOOD PRODUCTS

Elias, S., Komanowsky, M., Sinnamon, H. I. and Aceto, N. C. Converts collagen to food additives. Food Engineering 42 (1970) 125.

Whitmore, R. A., Jones, H. W., Windus, W. and Naghski, J. Preparation of hide collagen for food. J. Am. Leather Chemists Assoc. 65 (1970) 382-388.

POTATO UTILIZATION

Problems and Objectives

An increasing proportion of U. S. potato production is being processed into products such as chips, French fries and dehydrated potatoes. The welfare of the potato industry depends on continuing this trend. Processing of potatoes, however, poses several problems, the most important being disposal of potato wastes. The potato starch plants, which provide growers and processors with an outlet for cull potatoes, as well as other processors may be forced out of business by antipollution laws. Recovery of useful materials from potato wastes would help alleviate the potato waste problem and provide additional return to the processor. Predicting and controlling processing characteristics of potatoes is another area of concern. Potatoes placed in cold storage to extend the processing season require a lengthy reconditioning treatment which is not always successful. The control of the level of glycoalkaloid, a naturally occurring toxin in some breeding stocks, must be considered in the breeding of an improved processing potato. Research is also needed to provide new and improved processed potato products.

Major objectives of present research are:

1. Developing technology for recovery of useful materials, including proteins and amino acids, from potato waste.
2. Developing technology for evaluating potatoes for processing, controlling browning during processing, and predicting quality at time of consumer use.
3. Perfecting the explosion-puffing process to provide new and improved dehydrated potato products.
4. Identifying the major and minor glycoalkaloids of potatoes and developing rapid methods for their analysis.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED VEGETABLE PRODUCTS AND BY-PRODUCTS

Evaluation for Processing. The possibility of finding potatoes that can be chipped directly from 40°C storage was improved when 40% of 2600 seedling samples were found to produce satisfactory chips. Further studies were made of the role of sucrose synthetase (SS) in potato tuber cell development: its potential as an index of tuber

maturity, factors affecting its concentration, and its relation to sugar-starch balance during growth and storage. As previously noted, SS was high during tuber development and starch deposition and declined sharply to zero. Good and poor processing potatoes could not be distinguished at maturity by size, protein level, starch content or reducing sugar content. Tubers of poor processing potatoes accumulated more sucrose during rapid growth and at maturity showed 2.5X the sucrose in good processing potatoes.

Related research is in progress under a PL-480 grant at College of Agriculture, Krakow, Poland. Thirteen Polish varieties of potato tubers have been categorized into three general types based on their sugar transformations during varied periods of cold storage and reconditioning and they are as follows: a. low response to cold; b. fast sugar accumulation-rapid reconditioning; and c. fast sugar accumulation-poor reconditioning. Such varietal classification should allow the investigator to derive more specific data in subsequent studies. The information above is particularly relevant to U. S. interests since an American cultivar, Kennebec, was concomitantly grown and analyzed and serves as reference. Studies on the correlation of specific enzymatic activities versus storage and reconditioning phenomena are now in progress. Important enzymes in sugar metabolism such as sucrose synthetase, invertase, mylase, phosphorylase, and phosphoglucumutase are being evaluated. Collaboration of such data should rule out possible errors from data concluded from one specific geographic region.

Much of the variation in textural quality in French fried potatoes previously noted has been found to be related to large variations in the tensile strength in various areas of the raw tuber.

Dehydrated Potato Products. Methods were developed for the determination of volatile components associated with normal and off-flavors in potato flakes using gas liquid chromatography (GLC) headspace vapor analysis for low boiling compounds and GLC analysis of concentrates prepared by steam distillation for high boiling compounds. The identity and flavor contribution of these compounds were established by examination of GLC effluents and by comparison of flake chromatographic data with that obtained previously from explosion puffed potatoes for which peak identifications by GLC-mass spectrometry and retention time were available. Samples of commercial and experimental flakes were stored for 6 months and periodically evaluated by a trained taste panel and by GLC to relate flavor changes with specific volatile components. Flavor changes were associated with products of oxidative reactions. Volatile products of non-enzymatic browning reactions showed significant sample-to-sample variation initially and increased slightly during storage. Information derived from these and subsequent studies will be used to establish the chemical basis of flavor defects in potato flakes, to relate these defects to specific raw material, processing and storage variables, and to develop methods for extending product shelf life.

Using Fall crop, processing variety potatoes, Kennebec and Russet Burbank, obtained from the University of Maine experimental farm, explosion puffed dice were prepared for storage studies. Sugar levels were adjusted by storing at low temperatures and conditioning, to high (5.6 to 6.8% total reducing sugars (trs)) and low (1.4 to 1.8% trs) levels. Dice were prepared from both varieties at high and low sugar levels, and were stored in air and in nitrogen at different temperatures. At the end of six months storage (tests continue) it has been shown that -

- a. The use of nitrogen introduced into the batch gun with the super-heated steam lowers Strecker degradation products [isovaleraldehyde (IVA) or isobutyraldehyde (IVB)] to acceptable taste levels as measured by organoleptic tests.
- b. At temperatures of 73°F and lower, samples packed in nitrogen show no significant increase in IVA or IBA. Organoleptic tests confirm this.
- c. At 100°F, samples packed in nitrogen show progressive increase in IVA and IBA and become progressively darker in color.
- d. At 73°F, samples packed in air show little increase in IVA or IBA. A steady increase in hexanol is seen, indicating that the problem is one of oxidative rancidity.

A continuous explosion puffing system has been designed, based on the concept of separate heating and puffing zones. The valving devices for entry to and exit from the heating chamber have been made and have been tested for pressure integrity and mechanical action and are ready for use. The heating chamber is now in the final stages of fabrication and delivery is expected soon.

A dehydration-grinding-air classification procedure for production of potato starch without waste was developed.

PROTECT FOOD SUPPLIES FROM NATURALLY OCCURRING TOXINS

A GLC-mass spectrometer method has been developed for separation and detection of the aglycones of the potato glycoalkaloids. An improved extraction using glass extractor thimbles has rendered the extraction process quantitative. In 23 varieties of potatoes known to be involved in breeding studies, 18 glycoalkaloids have been reported. Several of them were not measured by the most generally used method for analysis of total glycoalkaloids. A 10-gram sample of highly purified solanine is being prepared for distribution as a standard to laboratories working in this field.

ALLEVIATION OF SOIL, WATER, AND AIR POLLUTION AND DISPOSAL OF WASTES

Potato Starch Factory Wastes. A simple reverse osmosis procedure was used to separate up to 80% of amino acids, sugars, and salts from the proteins in simulated potato starch factory waste. Up to 400 lb./day of a fraction containing 90% asparagine can be recovered easily from ion exchange eluates. Modification of ion exchange column design makes possible operation without prior protein precipitation, eliminating need for heat precipitation before ion exchange. Further process modifications increase organic acid concentration in the eluate from 4.3 to 7.8%. The fraction was found not to be useful as a detergent builder.

Protein recovery studies were completed by extending the concentration range to include full strength protein water. From protein water containing over 5% solids, i.e. undiluted juice, 99% of the protein was recovered by steam injection heating to 220°F followed by filtration; at the same concentration steam injection heating to 212°F or less, i.e., without superheating, yielded an 85% recovery. Using concentrations of 4.5% or less, acidification to a pH 5.5 or less was necessary even at 220°F to recover 99% of the protein by filtration, though the same recovery (99%) was obtained at 180°F. Without prior acidification at 220°F recovery decreased with dilution from 85% at 4.5% concentration to 75% at 2% concentration.

Cost studies revealed that evaporating the total effluent of potato starch factories has the best economic potential when compared with: (1) recovering protein only with biological oxidation of remainder, (2) recovering protein plus concentration of remaining solubles, (3) individual recovery of all valuables by the combined steam injection and ion exchange process, (4) biological oxidation of entire effluent.

Studies on evaporating protein water revealed that the concentrates above 50% are usually too viscous for practical handling. Preliminary attempts to evaluate a concentrate as a poultry feed ingredient revealed the practical impossibility of using a liquid concentrate; a dry product is required for this use. A dry product is also of interest as a fermentation nutrient.

Publications and Patents - USDA and Cooperative Program

NEW AND IMPROVED VEGETABLE PRODUCTS AND BY-PRODUCTS

- Lauer, F. and Shaw, R. A possible genetic source for chipping from 40°F storage. *Am. Pot. J.* 47 (1970) 275-278.
- Pressey, R. Changes in sucrose synthetase and sucrose phosphate synthetase activities during storage of potatoes. *Am. Pot. J.* 47 (1970) 245-251.
- Sapers, G. M., Sullivan, J. F. and Talley, F. B. Flavor quality in explosion puffed dehydrated potato. 1. A gas chromatographic method for the determination of aldehydes associated with flavor quality. *J. Food Sci.* 35 (1970) 728-730.
- Sapers, G. M. Flavor quality in explosion puffed dehydrated potato. 2. Flavor contribution of 2-methylpropanal, 2-methylbutanal and 3-methylbutanal. *J. Food Sci.* 35 (1970) 731-733.
- Sapers, G. M., Osman, S. F., Dooley, C. J. and Panasiuk, O. Flavor quality of explosion puffed dehydrated potato. 3. Contribution of pyrazines and other compounds to the toasted off-flavor. *J. Food Sci.* 36 (1971) 93-95.
- Shaw, R. and Turnquist, O. C. Potato variety trials, 1970 crop. *Valley Potato Grower* 19 (1971) 8.
- Zacharius, R. M., Krulick, S. and Porter, W. L. Concerning the constancy of the protein electrophoretic pattern of a potato tuber variety. *Am. Pot. J.* 48 (1971) 57-63.

ALLEVIATION OF SOIL, WATER AND AIR POLLUTION AND DISPOSAL OF WASTES

- Heisler, E. G., Krulick, S., Siciliano, J., Porter, W. L. and White, J. W., Jr. Potato starch factory waste effluents. I. Recovery of potassium and other inorganic cations. *Am. Pot. J.* 47 (1970) 326-336.
- Strolle, E. O., Cording, J., Jr., Aceto, N. C. and DellaMonica, E. S. "Recovering proteins from potato starch factory effluents. Progress and prospects", ARS 74-55, Proceedings 20th Annual Potato Utilization Conference, pp 71-76, January 1971.

VEGETABLE UTILIZATION

Problems and Objectives

The trend toward processing greater amounts of the vegetable production continues to accelerate under the impact of economic and social factors. Economically, utilization as processed, rather than fresh, vegetables provides a constant source of supply with less price fluctuation. Modern military feeding needs products with high bulk density which do not require refrigeration and are rapidly hydratable. Consumer preference is shifting to "convenience" foods. Therefore, the emphasis in research is on improvement in processing technology, particularly for dehydrated products.

The major objective of this research is to perfect the explosion-puffing process to provide dehydrated vegetables which rehydrate rapidly, cook in the same or less time than their fresh counterpart, and have good initial flavor and storage stability at room temperature.

Progress - USDA and Cooperative Program

NEW AND IMPROVED VEGETABLE PRODUCTS AND BY-PRODUCTS

Dehydrated Products. The batch explosion puffing process has been successfully applied to sweet potatoes, carrots, beets, rutabagas, celery, onions and peppers. Cost estimates have shown the batch process to be commercially feasible, but industry feedback has shown it to be apparent that a continuous puffing system is needed if commercial adoption of the process is to be achieved.

The operation of equipment that combined a pocketed rotating valve with EMN-designed rapid discharge device was successful with carrots but was unsuccessful with starchy and sugary vegetables due to accumulation of deposits on sealing surfaces. A continuous puffing system eliminating difficulty with sticky products has been designed and is being built.

In continuing cooperative research with Gilroy Foods Company, Gilroy, California, about 100 pounds of explosion puffed onion pieces were prepared in the batch puffing gun. This product is being distributed by Gilroy Co. to a cross-section of their customers. Thus far, reports on the product in uses where an instantly rehydrating onion is needed have been good.

DECIDUOUS FRUIT AND TREE NUT UTILIZATION

Problems and Objectives

It is essential that there be continued improvement in the quality of processed fruits if this commodity is to hold its own as an element in the national diet. Mechanical harvesting and other changes in the growing and harvesting of fruits create problems which must be met by improved processing methods. The development of new fruit varieties more suitable for various types of processing is an important factor in preserving markets for the fruit processing industry and protecting fruit growers against variations in price due to irregular yields from year to year.

Objectives of the research are:

1. To develop high quality dehydrated fruit products which may be reconstituted quickly, and which may be consumed as snacks.
2. To develop improved methods for the processing of mechanically harvested fruits and for maintaining the processing quality of fruits harvested by this method.
3. To evaluate the processing characteristics of new fruit varieties.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED FRUIT PRODUCTS AND BY-PRODUCTS

Cherries. Seven more on-farm tart cherry processing plants were built in 1970-71. Operating on ARS recommendations, 10% of the national crop was processed to a better product with an increase of \$450,000 in return to grower-processors. A new tart cherry product was developed and is being marketed. Work was continued toward obtaining approval for an abscission-promoting chemical (2-chloroethyl phosphonic acid) which will firmly establish practicality of mechanical harvesting of sweet cherries.

Identification of low- and high-boiling tart cherry aroma constituents is nearly completed. In a study of aroma development during processing, terpenes were found in the original fruit; esters, 2-hexenal, and benzaldehyde are formed enzymically after crushing, and the typical cherry pie flavor, largely non-volatile, appears after cooking.

Apples. Dewaxing of apples by 30 second exposure to isopropanol vapors removes about 1.7 lbs. crude wax per ton of apples. Residual isopropanol

in sauce and slices made from dewaxed, lye-peeled apples is about 35 ppm; a control sauce, made from mechanically peeled apples, contained 11 ppm naturally occurring isopropanol.

A new type harvester to pick apples from ground, described last year, shows potential for prunes, apricots, nuts and citrus.

Tomatoes. The isopropanol vapor procedure for dewaxing prior to lye peeling, developed at EMN with emphasis on apple peeling, is now in commercial usage with tomatoes in California.

Grapes. Several methods for bulk handling of machine harvested Concord grapes tested by the EMN-AERD-MSU team and one using obsolete cherry equipment were found satisfactory. Growers and processors saved about \$200,000 this year by their use.

Pears. Research on evaluation of processing characteristics of new Eastern pear varieties under a contract at Rutgers University, New Brunswick, New Jersey, has been completed. Seven of the 70 selections from the 1969 crop processed as canned halves were of a promising quality, with three having a Bartlett-like flavor. One, processed as puree, was superior to the commercial control.

Dehydrated Products. The batch explosion puffing process has been successfully applied to apples, pears and blueberries. The operation of the continuous puffing system in production of dried fruit products will be evaluated.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED FRUIT AND VEGETABLE PRODUCTS AND BY-PRODUCTS

- Levin, J. H., Tennes, B. R., Whittenberger, R. T. and Cargill, B. F.
Weight-volume relationships of tart and sweet cherries. Trans. ASAE
13 (1970) 489-490.
- Stabile, R. L., Turkot, V. A., Eisenhardt, N. H. and Cording, J., Jr.
Explosion-puffed apples are commercially feasible. Food Prod. 80
(1971) 80, 83, 86-88.
- Strolle, E. O., Cording, J., Jr., McDowell, P. E. and Eskew, R. K.
Effect of sucrose on crispness of explosion-puffed apple pieces
exposed to high humidities. J. Food Sci. 35 (1970) 338-342.
- Tennes, B. R., Levin, J. H. and Whittenberger, R. T. Development of a
mechanical pickup harvester for apples. U. S. Dept. Agr., Agr.
Res. Service, ARS 42-180 (1971) 13 pp.
- Whittenberger, R. T., Levin, J. H. Zoss, B. and Bukovac, M. J. Sweet
cherries: Abscission chemical aids mechanical harvesting. Canner/Packer
140(3) (1971) 16, 18, 20-21.

TOBACCO UTILIZATION

Problems and Objectives

Tobacco is grown on about a million acres, and in seven States typically provides more farm cash receipts than any other field crop. The most serious problem affecting the tobacco industry is the concern regarding effect on health.

Major shortcomings in developing a safer cigarette are the inadequacies of biological testing procedures and the failure to know which substances in tobacco and smoke should be removed. There is no practical procedure for determining which substances in tobacco and tobacco smoke are injurious to public health. Based on limited knowledge, some currently marketed cigarettes with charcoal and other filters apparently show selective reductions in certain properties in laboratory animals but cannot be considered a final solution. In addition, such cigarettes are deficient in flavor and aroma and meet with some consumer resistance. This problem requires a concerted effort to learn specifically what must be removed from cigarette smoke and how this can be done effectively without loss of desirable organoleptic properties.

Objectives of research are to:

1. Develop methods for determining which substances in tobacco and tobacco smoke are undesirable from standpoint of public safety, and
2. Develop technology for elimination of substances determined to be undesirable.

Progress - USDA and Cooperative Program

REDUCTION OF HAZARDS TO HEALTH AND SAFETY

Fractionation and Bioassay of Cigarette Smoke Condensate and Tobacco Leaf Samples. Continued large scale fractionation of tobacco smoke condensate for use in biological assaying has emphasized adsorption chromatography of the neutral fraction and gel filtration of the most polar neutral fraction. Bioassays, by application of test material to backs of mice over a 50-week period, indicate the presence or absence of biologically active constituents and provide guidelines for the selection of fractions for further subfractionation and testing. Further fractions of concentrated extracts of tobacco leaf (unburned cigarette tobacco) are also being tested in bioassays conducted under a contract with Health Research Inc., Buffalo, New York.

Similar bioassays of tobacco smoke condensate are in progress at Lexington, Kentucky, as part of the contract research program with the University of Kentucky. In these tests unfractionated tobacco smoke condensate are used to evaluate relative activity of different source materials, with recent emphasis on comparison of tobacco variety and method of curing.

Composition and Characteristics of Tobacco and Tobacco Smoke. Work has continued at EMN on the composition of tobacco smoke and its physiological and biochemical activities. A device for loading test cigarettes with additives and a system to measure concurrently the EMF and pH of cigarette smoke in solution have been developed. Electrometric measurements of the redox potential of smoke have indicated that almost all of the reducing capacity of smoke is generated during the first five puffs. Benzoquinone and sodium thiosulfate additives produced marked alterations in the oxidizing and reducing capacities of smoke, but required relatively high concentrations. Cellulose cottons treated to introduce sulfhydryl and/or ion-exchange groups were tested as filter additives, but their slow reactivity limits their effectiveness. Gel filtration was used to separate the most polar neutral fraction of cigarette smoke condensate into five sub-fractions of average molecular weight ranging from about 400 to more than 4000, and this separation procedure is being used to prepare fractions for bioassay.

A high molecular weight fraction was isolated from cured and fermented tobaccos. In salt solution this material had an average MW of 35,000; in the absence of salt it aggregated to a MW of over 500,000.

In research performed at EMN under a cooperative agreement with the Cigar Manufacturers Association of America, Inc., a detailed composition study was made of the odoriferous fractions in distillates from cigar butts. A substantial portion of the odorous constituents consists of pyridine bases. Gas-liquid chromatographic examination of steam distillate from butts showed the distillate contained approximately 4% each of indole and skatole. Several compounds not previously found in an earlier, preliminary study of cigar butt odor were identified, including ethyl mercaptan. This compound may play a significant role in the undesirable odor emanating from butts. Evidence was also obtained showing that the butt odor may be derived largely from the particulate matter condensed in the butt. Preliminary studies on adding an acid such as tartaric to the butt end of cigars indicates that it may be possible to alter butt odor to some extent without concurrently changing smoke flavor drastically by the use of cigar additives.

In biochemical investigations of harvested tobacco, studies on proteins in cured and fermented leaf were conducted for the first time using a new technique involving electrophoresis on Sephadex G-10. Peroxidase isozymic

patterns for different crop years of cured tobacco remained constant after a first-year decrease. This enzyme was absent from the leaf, stem and veins of fermented tobacco. The presence of a catalase was demonstrated in cured, stored and fermented tobaccos.

The results of organoleptic and chemical tests on two varieties of tobacco fermented at prevailing or controlled climate conditions have been reported from Tobacco Institute, Belgrade, Yugoslavia where research is being conducted under a PL-480 grant. For one variety the best smoking quality leaf was obtained when fermented under seasonal temperatures and humidity, but for other characteristics best results were obtained under a controlled climate at 35°C. The second variety was considered to be best in both evaluations when fermented at 25°C. Based on one year's experiments, the investigators emphasize that the results are not conclusive.

At the University of Kentucky Research Foundation, Lexington, several composition studies are conducted under contract. In a study of higher-boiling heterocyclic nitrogen compounds, separation techniques, such as solvent partition, thin layer chromatography, column chromatography, and gas chromatography, were applied to both the basic and neutral fractions of smoke condensate. Isolated components were characterized by mass spectrometry and nuclear magnetic resonance. Additional compounds identified in the basic fraction included anabasine and N-methylanabasine. No new compounds were identified in the neutral fraction.

Studies were continued on the analysis of nitrosamines by reduction to hydrazines and conversion of the latter to 9-anthraldehyde hydrazones. Mass spectra of the hydrazones of 9-anthraldehyde provide a promising tool for the identification of the hydrazones and their parent nitrosamines. All the 9-anthraldehyde hydrazones have a base peak at m/e 203. The peak corresponding to the parent ion ranges from 0 to 89% of the base peak. An important characteristic of the hydrazone spectra is that 9-anthraldehyde has no ion at m/e 203. Since this aldehyde is the most probable contaminant stemming from separation of the hydrazones by column - or thin layer - chromatography, its interference can be ruled out in the identification of the hydrazones. Polarographic reduction of dimethylnitrosamine to dimethylhydrazine by the method of Barkemeyer was confirmed.

Twelve trace elements - La, As, Br, Cr, Se, Ag, Zr, Sc, Zn, Fe, Co, and Sb - have been determined in Burley tobacco leaves, stalks, ashes, and 19 different brands of cigarettes. Filter cigarettes are much more effective in preventing the transference of these trace elements from cigarettes into smoke than the nonfilter cigarettes.

Pyrolysis Products. In studies related to processes occurring in a burning cigarette, a number of naturally occurring materials were pyrolyzed and the products thus generated were characterized. At 840°C and in an atmosphere of nitrogen, carbohydrates gave rise to simple aromatic hydrocarbons, furan derivatives, simple phenols and polynuclear aromatic hydrocarbons. Proteins under the same conditions also generated aromatic hydrocarbons and phenols, and in addition produced aromatic nitriles, pyridine derivatives, quinoline, isoquinoline and aniline. Amino acids, on pyrolysis, behaved similarly to proteins, and in some cases gave rise to products that were structurally related to the amino acids pyrolyzed (e.g. tryptophan yielded indole and skatole, inter alia). Fatty materials at 800°C and higher generated aromatic hydrocarbons predominantly. However, at lower temperatures (e.g. 650°C), fatty acids, glycerides and long chain hydrocarbons gave rise to a homologous series of alkenes (monoenes), and in the case of the glycerides a homologous series of alkanoic acids, as well. The degree to which some of the substances pyrolyzed gave rise to the biologically active benzo(a)pyrene was also determined. Significantly, the pyrolyzates from protein contained no benzo(a)pyrene.

A comparison of pyrolyzates from fermented tobacco revealed only minor qualitative differences, while quantitative differences were consistent with known alterations in leaf components resulting from fermentation.

In contract research at the University of Kentucky, the yields of the important components of the neutral and basic fractions from the pyrolysis of methionine, cysteine, cystine, homocystine and methionine sulfone in nitrogen were determined. Methionine was also pyrolyzed in air and the pyrolyzate separated into major fractions. The yields and identity of the important components of the neutral and basic fractions were determined. In addition to the identification of compounds which were present in the pyrolyzates, it was established that twenty-three sulfur compounds of various types are not present in significant amounts in the pyrolyzates of the sulfur amino acids studied. In view of the reported cocarcinogenicity of sulfur dioxide with benzo(a)pyrene, it is pertinent to the tobacco and health problems that no significant amounts of sulfur dioxide were formed during pyrolysis of methionine in air.

Thermal decomposition of the sucker-inhibiting agent, N,N-dimethyldodecylamine, was studied in air and nitrogen atmospheres. Although the pyrolyses in either atmosphere produced the same products, variations in the quantities were observed. Some of the substances identified were hydrogen cyanide, aromatic nitriles, nitrogenous bases, aromatic hydrocarbons and benzo(a)pyrene. Carbon dioxide was the only substances produced in the air pyrolysis which was not observed in the nitrogen pyrolysis. Phenol was produced during the pyrolysis of maleic hydrazide in air. These experiments suggest that residues of sucker-inhibiting agents can contribute to the production of toxic and carcinogenic materials during the burning of tobacco.

Experimental Cigarettes and Analytical Methods. In supporting research at the University of Kentucky, the selecting of uniform cigarettes was improved by installation of a pressure drop testing instrument. Also, a slight modification of the Questor tobacco cutting machine was made which improved the cutting of leaf into shreds and minimized loss of fine tobacco particles. The cigarette preparation laboratory is enabling research workers to obtain maximum information from relatively small samples of tobacco.

The procedure for the analysis of smoke from treated tobacco was modified in order to fit into the routine smoke analysis laboratory facilities. All analyses now are being determined from 5 cigarettes using Cambridge filter pads rather than the trapping of smoke from 20 cigarettes in a dry ice-acetone bath. Columns have been prepared from alumina containing rare earth oxides and they are presently being screened for their usefulness in the separation of polynuclear aromatic hydrocarbons.

A procedure was established for the dilution of fresh cigarette smoke condensate for bioassay experiments. The concentration of these preparations is based on the residue weight which would be obtained by the conventional acetone drying procedure. Development of a procedure for the preparation of the water-soluble fraction (WS) of whole smoke by direct collection in water was completed. A fractionation scheme was developed which gives four primary fractions of whole condensate by the use of solvent partition. The enriched aromatic fraction obtained from this fractionation procedure is currently being used in biomedical investigations.

Development of New Bioassay Methods. In contract research at the University of Kentucky Research Foundation, Lexington, studies continue on biological systems and on their response to tobacco constituents in evaluating their use as a bioassay procedure to replace or augment the method of repeated applications to backs of mice over a 50-week test period.

Staphylococcus aureus has proved to be an almost ideal system in which smoke and smoke components can be tested. Genetic analysis and mutant selection techniques allow study of defects in the formation of the membrane-bound respiratory system. Vitamin K₂ isoprenologues, carotenoids, and phospholipids have been isolated, characterized, and their metabolism studied in detail. With this test system fresh smoke and benzo(a)pyrene have been shown to effect the synthesis of the respiratory system (primarily cytochrome oxidase o) and the hydroxylation of δ -carotene in the formation of rubixanthin. Benzo(a)pyrene effected an increase in the total phospholipids and an inhibition on the electron complex formation. Tests of other smoke components and aged smoke preparations were not effective in this system.

Investigations were carried out to study the response of plant tissues to tobacco smoke constituents. Experimental materials used for this study were callus tissues obtained from haploid and diploid plants of Nicotiana tabacum and the intact plants of Lemna gibba G3. Water-soluble extract of tobacco smoke condensate (WS) induced vegetative buds on callus derived from haploid tobacco plants, a response similar to that obtained by the combination of kinetin and 3-indoleacetic acid. However, no morphogenesis was observed on the green callus derived from diploid tobacco plants in the presence of WS. The effects of WS, enriched polynuclear aromatic hydrocarbon fraction of tobacco smoke, and benz(a)anthracene were studied in M and 1/3 strength Hutner's media on the growth and flowering of Lemna gibba G3 in the presence and absence of ethylenediamine-di-o-hydroxy-phenylacetic acid. These smoke constituents had a marked inhibitory effect on flowering but relatively little influence on the multiplication rate. The effects on growth and flowering indicated that plants are possible experimental materials for bioassay studies.

Dose-response studies were initiated for the water-soluble extract of the IAI (low nicotine) cigarette smoke condensate. Preliminary data indicate that the aqueous extract of IAI is much more ciliostatic at higher concentrations (20 to 40 mg/ml) than a comparable extract from the Kentucky Reference Cigarette smoke condensate (IRI). At lower concentrations (2.5 to 15 mg/ml) the ciliostatic activities were essentially equal. The ciliostatic effect of IRI could be nullified by the addition of certain levels of cysteine to the aqueous solution. Attempts are being made to develop a quantitative method, involving liquid scintillation counting, for the determination of the extent of inactivation of tracheal tissue by cigarette smoke.

Studies were undertaken to determine the effect of the water soluble fraction (WS) from whole smoke of IRI cigarettes on the respiratory activity of intact mitochondria as well as the submitochondrial particles prepared from rat liver. WS exhibited a strong inhibitory effect on the oxidation of various compounds by intact mitochondria and submitochondrial preparations. Although commercial nicotine as well as phenol when used individually at relatively high concentrations could not account for the inhibitory effect of WS, their combined effect in causing inhibition of respiratory enzymes has not been ruled out.

Intravenous injection of selected polynuclear aromatic hydrocarbons effected marrow cell chromosome breakage in rodents. A correlation between the ability of these compounds to produce chromosome breaks and to induce mammary neoplasms was demonstrated. Administration of an enriched aromatic fraction of cigarette smoke condensate, however, did not produce a comparable response. These animals have remained healthy and tumor-free for 10 months. Chromosome studies, 24 hours following a single intravenous injection, indicated an incidence of chromosome breakage comparable to that noted in a control group.

Graded doses of the aqueous extract of cigarette smoke condensate (AECSC) prepared from the University of Kentucky Reference Cigarette, IRI, were administered to female (Sprague-Dawley strain) rats, age 30 days, for four weeks. A concentration of 2 mg/ml induced profound weight loss which cannot be explained by dehydration alone; concentrations of 0.5 and 1.0 mg/ml retarded normal weight gain, whereas concentrations of 0.125 or 0.25 mg/ml had no significant effect on body weight. A concentration as high as 16 mg of the nonaqueous fraction of cigarette smoke condensate, prepared from IRI cigarettes was tolerated by female rats given a subcutaneous injection on alternate days. It has not induced tumors in the test animals during the observation period of five months. The subcutaneous injection of 2 or 4 μ g benzo(a)pyrene on alternate days for 30 doses induced sarcomata at the site of injection. Tumor incidence was higher when AECSC 0.25 mg/ml was given in 5% sugar solution as drinking fluid. No tumors have been detected in rats injected with 1 μ g-doses.

The effects of microbial flora and tobacco smoke inhalation on blood pO_2 , pCO_2 , cardiac output, and regional blood flow were determined in conventional and germfree rats. Acute tobacco smoke inhalation caused the following changes: Cardiac output and regional blood flow (studied to date only in conventional rats) showed a substantial depression, with the exception of blood flow in the bronchial circulation and in some endocrine glands. Blood pO_2 and pCO_2 levels (carried out both in conventional and germfree rats) indicated that tobacco smoke exposure did not impair the oxygenation of arterial blood. When smoke-exposed germfree rats were compared to normal germfree rats, little, if any, differences were found in pO_2 among any of the blood samples. In conventional rats, smoke exposure left arterial pO unchanged, but depressed significantly this value in venous blood. The presence of the microbial flora in the animal host appears to elevate various components of blood circulation, while tobacco smoke inhalation depresses them. The microbial flora and acute exposure to tobacco smoke, individually or in combination, do not interfere with the process of oxygenation of the blood in the course of its passage through the lungs.

The distribution and metabolism of benzo(a)pyrene (BAP) in rats, as affected by phenol and phenanthrene, were studied. After oral administration tritiated BAP was found to be concentrated in the mammary glands and perirenal fat. It is proposed that 6-hydroxymethyl-benzo(a)pyrene is the proximate carcinogen of BAP.

Studies on the absorption of carcinogens from the gastrointestinal tract of rodents showed that BAP is absorbed and also converted to a more polar compound. In mammary gland and in adipose tissue a linear increase in carcinogen dose resulted in an exponential increase in tissue concentration.

Several studies designed to further clarify the enhancing factor present in methylcholanthrene (MC) sarcoma (Sa) homogenate were performed. It is now evident that proximity of the MCSa homogenate to the primary carcinogen, MC, is necessary to produce the enhancing effect. Interestingly, a mixed homogenate of spleen, kidney and lung taken from tumor bearing animals seems to have identical enhancing activity as MCSa homogenate, whereas other organs did not. A soluble antigen is indicated to be present in MCSa. Multiple attempts have been made to induce a true tobacco smoke condensate (TSC) tumor. Thus far, however, no tumors have formed. The development of a TSC tumor and the delineation of the tumor's characteristics relative to MCSa should be of considerable significance in furthering our understanding of tobacco products carcinogenesis.

Publications and Patents - USDA and Cooperative Programs

REDUCTION OF HAZARDS TO HEALTH AND SAFETY

- Bailey, D. G. An eluent programmer for column chromatography. J. Chromatog. 50 (1970) 137-139.
- Benedict, R. C., Lakritz, L., Strange, E. D. and Stedman, R. L. Redox characteristics of cigarette smoke. Chem. Ind. (London) (1970) 800-802.
- Bock, F. G., Swain, A. P. and Stedman, R. L. Composition studies on tobacco XLI. Carcinogenesis assay of subfractions of the neutral fraction of cigarette smoke condensate. J. Natl. Cancer Inst. 44 (1970) 1305-1310.
- Bruzel, A. R. and Schmeltz, I. Qualitative studies of pyrolysis of bi-phenylamine, carbazole, acridine and nicotinamide at 1050°C. Tob. Sci. 15 (1971) 44-46.
- Higman, E. B., Schmeltz, I. and Schlotzhauer, W. S. Products from the thermal degradation of some naturally occurring materials. J. Agr. Food Chem. 18 (1970) 636-639.
- Kushnir, I., Barr, P. A. and Chortyk, O. T. An improved method for volatile phenols. Anal. Chem. 42(13) (1970) 1619-1621.
- Miller, R. L. and Stedman, R. L. Alkylphenols and aryl nitriles in a biologically active neutral subfraction of cigarette smoke condensate. Phytochemistry 10 (1971) 1135-1140.
- Rees, E. D., Majumdar, S. K. and Shuck, A. Changes in chromosomes of bone marrow after intravenous injections of 7,12-dimethylbenz(a)anthracene and related compounds. Proc. Nat. Acad. Sci. 66 (1970) 1228-1235.
- Schepartz, A. I. Adaptors for the aerograph pyrolyzer. Gas-Chrom. Newsletter 11(4) (1970) 8.
- Schepartz, A. I. and Schepartz, R. C. Respirometer for student use. Amer. Biol. Teacher 33 (1971) 107.
- Schlotzhauer, W. S., Schmeltz, I. and Osman, S. F. Evidence for the origin of monoalkenes in cigarette smoke. Chem. Ind. (London) (1970) 1377-1378.
- Schmeltz, I. Nicotine and other tobacco alkaloids, in Naturally Occurring Insecticides, pp 99-136 (1971) Marcel Dekker, Inc., New York.

MAPLE SAP AND SIRUP UTILIZATION

Problems and Objectives

Maple sirup producers are largely full-time small farmers, in an area encompassing 14 States from Maine to Minnesota and south to Virginia. These producers are utilizing less than 3% of the available sugar maple trees for sap production. Extensive stands of untapped trees are located in agriculturally depressed areas and since, under proper conditions, maple sirup can be a six-week seasonal crop not in competition with other farm activities and with a per acre value equal to or exceeding that of other farm products, these trees represent a good potential source of cash income in these areas. The production of maple specialty items is another potential source of cash income for the farmer or producer. Sustained effort is required to obtain the information needed so that all operations for the production of high-quality maple sirup and other maple products can be conducted in a predictable, efficient manner.

Major objectives of the research are:

1. Improving technology and reducing cost of sap collection and processing.
2. Improving quality of maple sirup and other maple products.
3. Developing new maple products.

Progress - USDA and Cooperative Programs

NEW AND IMPROVED FOREST PRODUCTS

Processing technology. Pilot-scale tests with simulated maple sap confirmed that reverse osmosis units with tubular configuration could be used to concentrate maple sap. These units are more easily sanitized and cleaned; late-season slime accumulation had been found to reduce the efficiency of the spiral-wound modules field-tested in earlier seasons. As previously reported, flavor precursors are retained in the concentrate, and maple flavor is developed on boiling the concentrate to produce maple sirup. A commercial demonstration of the process has been made in the Wisconsin maple area, and a Vermont cheese producer is considering combining seasonal maple sap processing with his proposed reverse osmosis cheese whey operation.

Improving quality of sirup. An inexpensive, easily constructed unit was designed and tested for the ultraviolet irradiation of maple sap.

Effective pasteurization of sap at 600 gal./hr. was attained using a 4-light unit, costing considerably less than commercially available water purifier units. Previous studies had shown that reduction of bacterial growth resulted in a higher grade sirup.

Further improvements in the resazurin test for bacterial contamination of maple sap have been made. Use of an opaque test solution improves the accuracy of the test, since a permanent end-point standard can be used.

Possible interference with the determination of formaldehyde in maple sirup has been examined. The method, as applied to maple sirup, has been found to be very specific for formaldehyde, though some improvement is needed in the details of the procedure to improve reproducibility.

IMPROVED INCOME OPPORTUNITIES IN RURAL COMMUNITIES

Utilizing Sugar Maple Resources. Under a contract with West Virginia University, Morgantown, sugar maple resources are being evaluated to determine the feasibility of locating a central processing plant in West Virginia. In five of 19 counties surveyed, possible sites were located for further study. To date, three counties (Grant, Tucker, and Preston), show potential to support maple sirup manufacturing operations. A significant portion of the potential is in Monongehela National Forest, and a successful maple industry in this State will probably depend on the use of these trees.

Publications and Patents - USDA and Cooperative Programs

NEW AND IMPROVED FOREST PRODUCTS

- Kissinger, J. C. Sanitation studies of a reverse osmosis unit used for concentration of maple sap. J. Milk & Food Technol. 33 (1970) 326-329.
- Kissinger, J. C. Estimating bacterial populations in raw and simulated maple sap by a modified resazurin test. J. Assoc. Offic. Anal. Chemists 54 (1971) 27-29.
- Kissinger, J. C. Report on maple products. J. Assoc. Offic. Anal. Chemists 54 (1971) 318.
- Underwood, J. C. Determination of formaldehyde in maple sirup: Variation of the blank. J. Assoc. Offic. Anal. Chemists 54 (1971) 30-32.
- Underwood, J. C. Effect of heat on the flavoring components of maple sirup: A preliminary study by gas chromatography. J. Food Sci. 36 (1971) 228-230.
- Underwood, J. C., Kissinger, J. C. and Bell, R. A. Field tests of the reverse osmosis unit for concentrating maple sap. Natl. Maple Syrup Digest 10(1) (1971) 9-14.

